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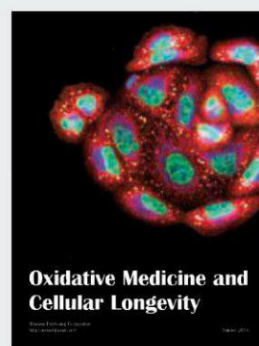
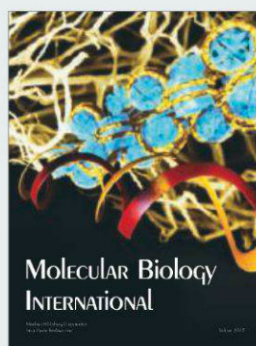
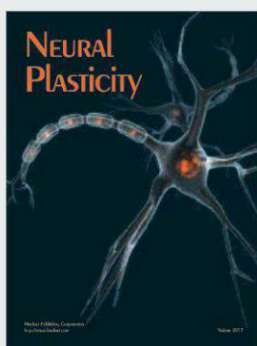
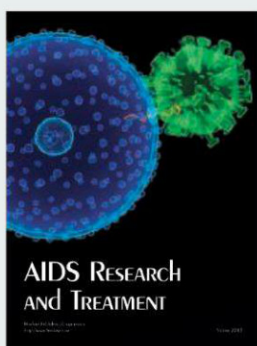
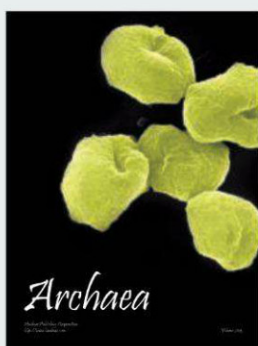
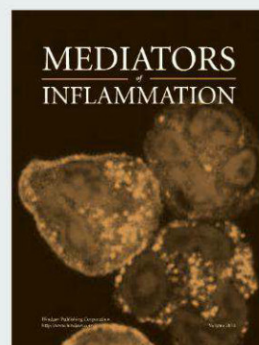
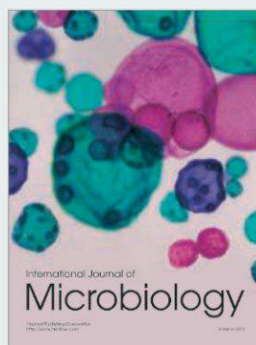
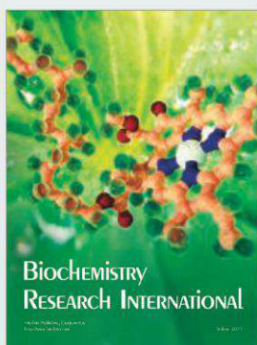
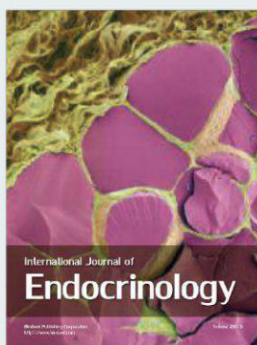
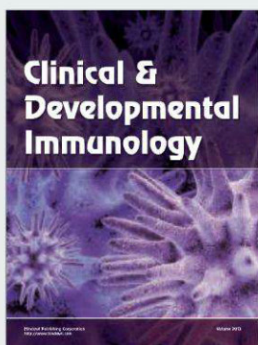
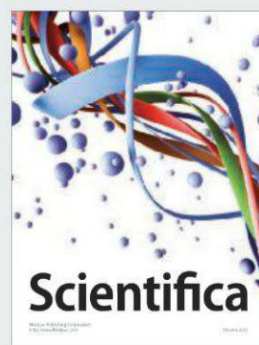
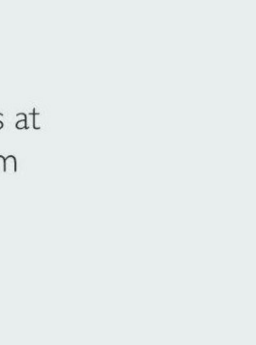
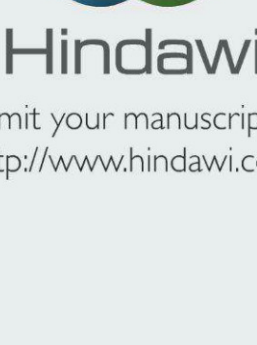
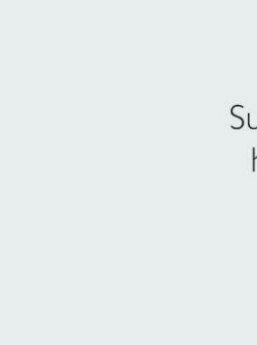
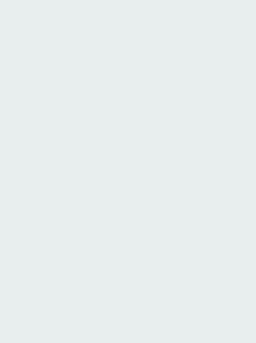
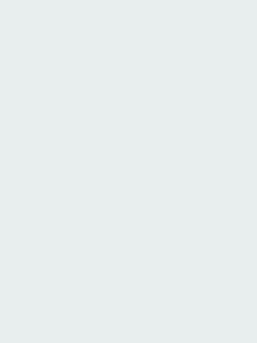
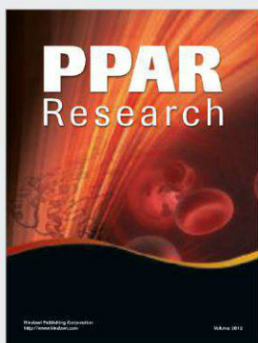
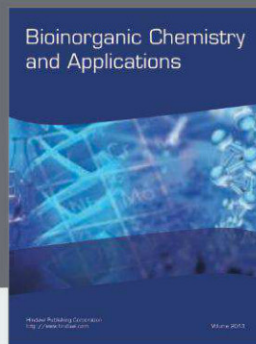
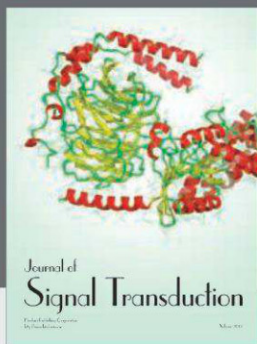
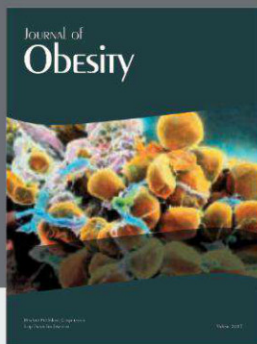
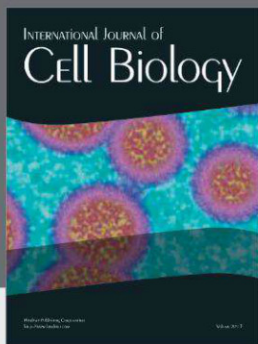
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CRI137B	Recombinant Human IL-15	10 µg
CRI162B	Recombinant Human IL-17	25 µg
CRI172B	Recombinant Human IL-21	10 µg
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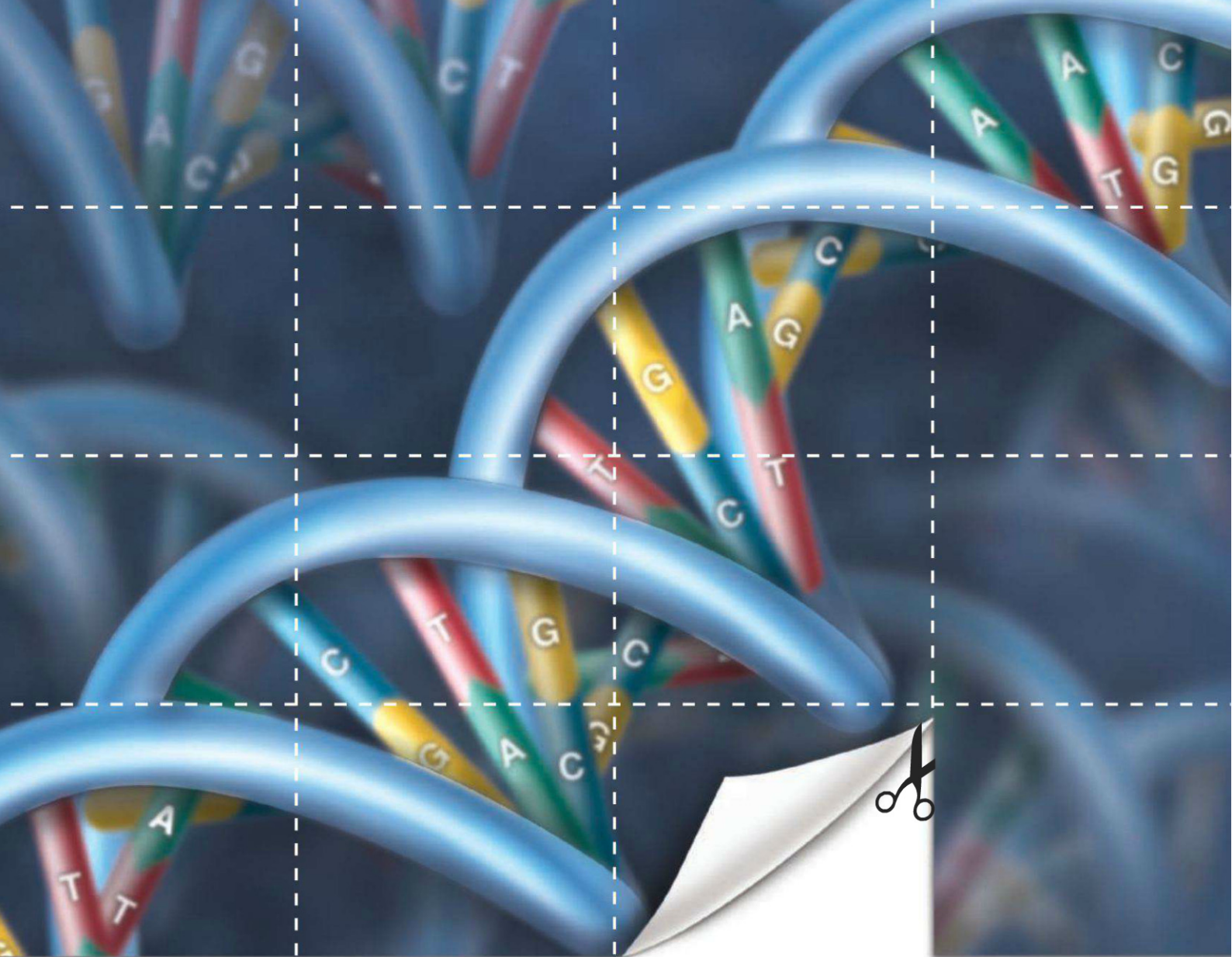


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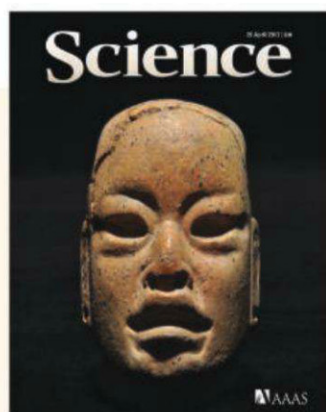
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COVER

A carved stone head (height: 6.8 centimeters) excavated from the lowland Maya site of Ceibal, Guatemala (around 400 BCE). This piece was probably part of a full-figure statuette. Its carving in the typical Olmec style attests to the importance of interaction with other groups for the early development of lowland Maya civilization. See pages 417 and 467.

Reconstruction: Daniela Triadan; Photo: Takeshi Inomata

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Saturn's Meteoroid Crash

During Saturn's equinox in 2009, when the Sun illuminated its rings edge-on, images taken by the Cassini spacecraft showed dust clouds appearing as bright streaks above the rings. Similar streaks were detected in 2005 and 2012 when Cassini observed the C ring at close range. **Tiscareno *et al.*** (p. 460) suggest that the cause of each observed feature is likely to be the impact of a stream of recently disrupted material originating from a meteoroid impact onto the ring and derive the influx rate of meteoroids at Saturn.

Hot Enough to Melt Iron

Earth's core is divided into a fluid outer core and a solid inner core, both composed predominantly of iron at extremely high pressures and temperatures. The boundary between these two regions is largely controlled by the melting point of iron at ~330 GPa, which in turn influences heat transfer and geodynamo generation. **Anzellini *et al.*** (p. 464, see the Perspective by **Fei**) compressed iron in a laser-heated diamond anvil cell, tracking its structure and texture by using time-resolved x-ray diffraction as the pressure increased to 200 GPa. The melting curve suggests the possibility of high heat flux and partial melting at the core-mantle boundary.



Double Vision

Vaterite is the least stable form of anhydrous crystalline calcium carbonate. While rarely found in geological contexts, it is an important biological precursor and occurs as a minor component in the shells of

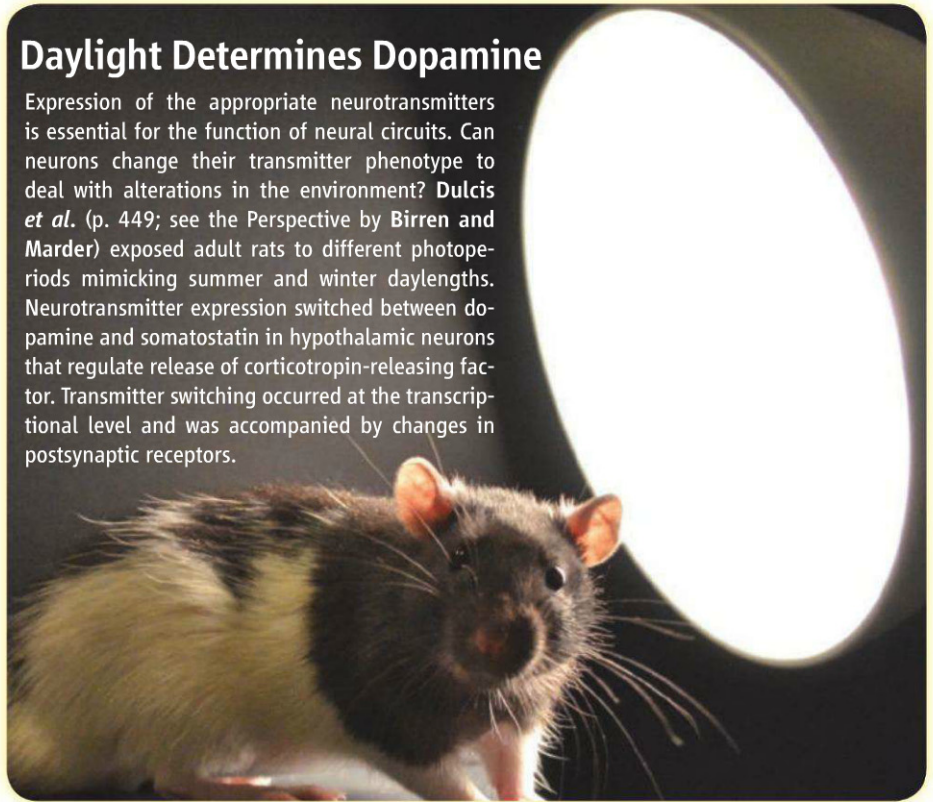
some organisms. The crystal structure of vaterite has long been debated with no model able to explain all the experimentally observed diffraction spots. **Kabalah-Amitai *et al.*** (p. 454) show that vaterite contains two coexisting crystallographic structures that form a pseudo-single crystal.

Early Mayan Architects

Lowland Mayan civilizations began to construct dramatic pyramids beginning about 900 BCE. The cultural origins of these civilizations have been uncertain, particularly the connection to earlier Olmec cultures along the Gulf of Mexico. **Inomata *et al.*** (p. 467; see the cover; see the News story by Pringle) describe excavations and extensive radiocarbon dating at the early Mayan city of Ceibal in Guatemala. The site shows incipient plaza and pyramid construction beginning

Daylight Determines Dopamine

Expression of the appropriate neurotransmitters is essential for the function of neural circuits. Can neurons change their transmitter phenotype to deal with alterations in the environment? **Dulcis *et al.*** (p. 449; see the Perspective by **Birren and Marder**) exposed adult rats to different photoperiods mimicking summer and winter daylengths. Neurotransmitter expression switched between dopamine and somatostatin in hypothalamic neurons that regulate release of corticotropin-releasing factor. Transmitter switching occurred at the transcriptional level and was accompanied by changes in postsynaptic receptors.



before those seen in the lowlands or Olmec areas, suggesting a broader cultural exchange through to the Pacific Coast as Mayan cultures evolved.

Where Parkin Parks

Damaged mitochondria are removed from cells in a process known as mitophagy. Failure of this quality-control mechanism contributes to Parkinson's disease. When damaged mitochondria lose membrane depolarization, the protein kinase, **PINK1**, accumulates on the mitochondrial surface, recruits Parkin, and promotes mitophagy. **Chen and Dorn** (p. 471) describe another component of this process, mitofusin 2, which appears to function as the receptor for Parkin on the surface of damaged mitochondria.

Viruses and Congenital Disorders

Mutations in genes involved in α -dystroglycan O-linked glycosylation result in posttranslational modifications associated with the congenital disease Walker-Warburg syndrome (WWS). This cellular modification is also required for efficient Lassa virus infection of cells. **Jae *et al.*** (p. 479, published online 21 March) screened for genes involved in O-glycosylation that affected Lassa virus infection and identified candidates involved in glycosylation. Individuals from different

pedigrees exhibiting WWS had unique mutations among genes identified in the genetic screen. Thus, comprehensive forward genetic screens can be used to define the genetic architecture of a complex disease.

Animal Culture

Cultural transmission of information occurs when individuals learn from others with more experience or when individuals come to accept particular modes of behavior as the local norm. Such information transfer can be expected in highly social or long-lived species where contact and time for learning are maximized and are seen in humans (see the Perspective by **de Waal**). Using a network-based diffusion analysis on a long-term data set that includes tens of thousands of observations of individual humpback whales, **Allen *et al.*** (p. 485) show that an innovative feeding behavior has spread through social transmission since it first emerged in a single individual in 1980. The "lobtail" feeding has passed among associating individuals for more than three decades. **Van de Waal *et al.*** (p. 483), on the other hand, used a controlled experimental approach in vervet monkeys to show that individuals learn what to eat from more experienced individuals within their social group. Not only did young animals learn from observing older animals, but immigrating males switched their food preference to that of their new group.

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Shinya Yamanaka is a professor and director at the Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan, and is the recipient of the 2012 Nobel Prize in Physiology or Medicine. E-mail: yamanaka@cira.kyoto-u.ac.jp

Young Researchers in Japan

NEXT MONTH IN TOKYO, ASIA'S LARGEST BIOTECHNOLOGY EVENT WILL TAKE PLACE—THE International Bio Technology Exhibition and Conference. Japan's traditional strength in both biology and technology makes it a very appropriate site for this forum. But over the long term, Japan's science and technology future will require that our nation place a much stronger focus on encouraging risk taking and independence for promising young scientists.

Currently, for a young researcher in the sciences to become an independent group leader at an academic institution requires that he or she experience science in its highly competitive international context, thereby acquiring a broad range of ideas and skills, and a global network of colleagues. For a young Japanese scientist, this requires boldness in one's career path, often involving graduate and/or postdoctoral training abroad. But many talented young Japanese today express great anxiety about leaving Japan for such training. The fear of going abroad causes many students to forgo a career in academic science, and it even prevents talented youth from entering Ph.D. programs. Therefore, there is a need to establish a variety of career paths for graduate students and other young researchers that enable them to feel secure enough to concentrate on their studies and training, whether they remain in Japan or benefit from being in an international arena.

The government of Japan has been seeking ways to support young scientists. A system of awards and scholarships for graduate students was instrumental in increasing the number of science graduate students by 2.8-fold over the past two decades. To keep graduate students on an academic career trajectory, the government has improved research assistant and teaching assistant opportunities. Japan has also established internships in the industrial arena to smooth career paths outside of academia. The government also implemented a research administration system at selected universities where postdoctoral fellows can be employed as administrative officers on the basis of their background in science.

Japan's Ministry of Education, Culture, Sports, Science and Technology (MEXT) is working to address two persistent structural problems that have long restrained the performance of Japanese science.* One issue is Japan's vast underutilization of female talent. In 2010, Japan employed 13.8% female researchers compared to 37.9% for the United Kingdom (as of 2008). As one example, MEXT is providing special funding for universities that help researchers who need childcare. The second major problem is a lack of independence for beginning researchers that hinders creativity. To establish new environments that permit young scientists to conduct research independently, the Japanese government has been developing a tenure track system that allows selected postdoctoral fellows to become independent principal investigators at participating institutions; in 2011, over a hundred such tenure track positions were supported.

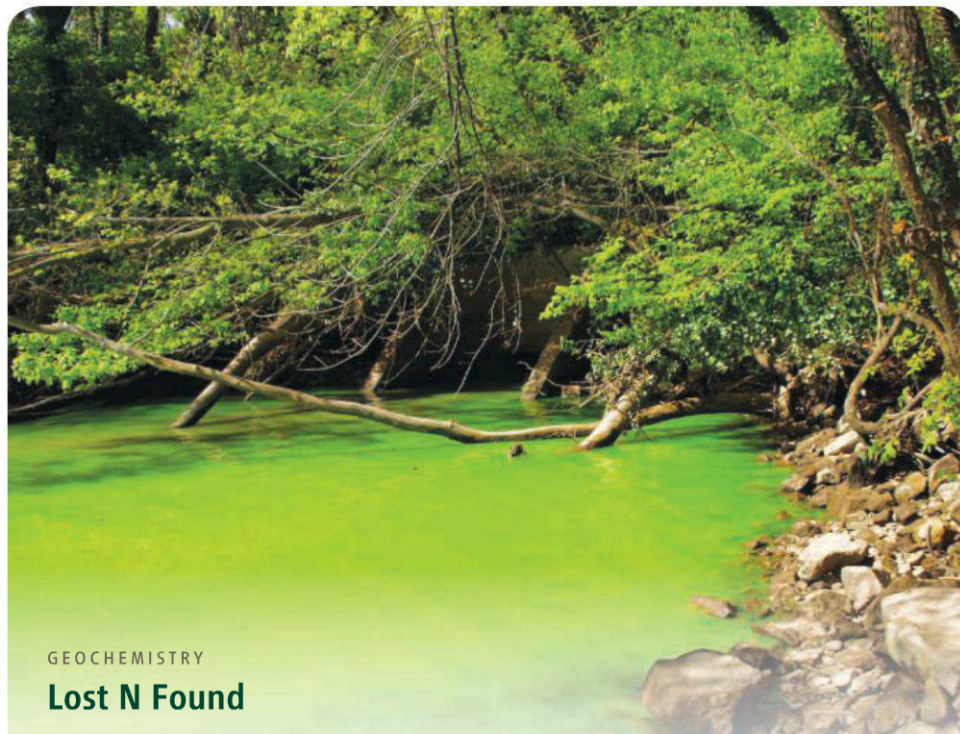
Because a vast number of researchers are expected to retire over the next few years, Japan has a unique opportunity to restructure its academic environment. To make the transition, there is an urgent need to introduce an evaluation scheme for principal investigators that is more transparent, reliable, and fair—based only on the perceived excellence of the individual involved. By creating a system that is completely merit-based, we can increase diversity by opening doors to young scientists with innovative and independent ideas, as well as to female researchers and to those who have developed their scientific careers abroad. To avoid the inevitable academic biases inside Japan, a new evaluation scheme should be instituted that incorporates expert international reviewers. The most important thing for building a solid foundation for the development of Japanese science is to support the success of all of our most talented researchers—whether upcoming or established, female or male, Japanese-born or not. The establishment of a much more transparent, unbiased, and reliable evaluation scheme for the recruitment of principal investigators lies at the heart of this critically needed change.

—Naoki Nagata and Shinya Yamanaka

10.1126/science.1239057

*www.mext.go.jp/component/english/_icsFiles/afieldfile/2013/01/15/1329766_14.pdf





GEOCHEMISTRY

Lost N Found

Humans are adding new reactive forms of nitrogen (N) into the environment, which has the potential to cause a range of problems, including eutrophication and the formation of dead zones in lakes and coastal waters. The microorganisms responsible for these “N loss” pathways, known as denitrification or anaerobic ammonium oxidation (anammox), often reside in sediments, but a variable and limiting supply of organic matter makes it difficult to determine which reaction dominates. Babbin and Ward set out to address this problem in the lab by constructing a series of mesocosms out of sediments from the Chesapeake Bay, United States, with large amounts of organic matter added to some of the columns. Over 7 weeks of incubation and monitoring, the proportion of each pathway was dictated more by the relative N content of the organic matter than by the total organic matter content. Moreover, the microbial communities in the sediments were able to quickly adjust to high N loading, such as sewage effluent or fertilizer runoff, so that most of the reactive N would be removed from the ecosystem and potentially released back to the atmosphere. — NW

Environ. Sci. Technol. 10.1021/es304842r (2013).

IMMUNOLOGY

SREBPs for T Cell Expansion

Responding to infections is energetically demanding, especially for cytotoxic CD8⁺ T cells. Once these cells recognize an infection, they blast, which requires lipid biosynthesis, and then undergo metabolic reprogramming so that they rely primarily on glycolysis to meet their high energetic demands. The specific mechanisms that allow for this transition are not well elucidated. Kidani *et al.* identify a role for the sterol regulatory element-binding proteins SREBP1 and SREBP2, transcription factors that regulate lipid homeostasis, in this process. The expression of SREBPs is up-regulated in response to T cell activation and is required for the induction of a lipid synthesis program. CD8⁺ T cells from

mice with a T cell-specific deficiency in SREBPs exhibited poor blastogenesis and proliferation upon activation, altered lipid homeostasis, and did not undergo the typical activation-induced metabolic reprogramming. Impaired responses of SREBP-deficient mice to infection with lymphocytic choriomeningitis virus underscored the physiological relevance of this pathway. — KLM

Nat. Immunol. 14, 10.1038/ni.2570 (2013).

CELL BIOLOGY

Haste Makes Waste

mTORC1 is a protein kinase complex that regulates many biological processes, including cell growth and proliferation, and that has a primary role in the control of protein synthesis. Inhibition of mTORC1 increases life span, and partial

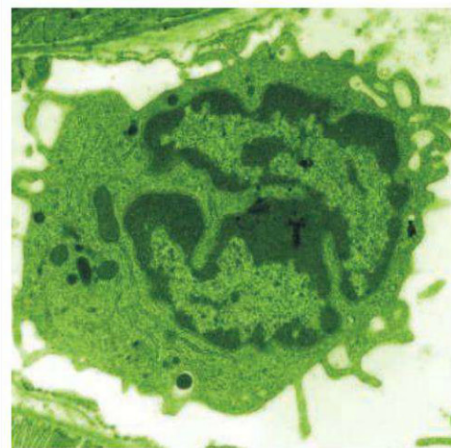
inhibition of translation mimics the effect on life span, indicating that effects of mTORC1 on life span might be related to effects on protein synthesis. Conn and Qian present a mechanism by which reduced rates of protein synthesis might extend life span. They found essentially that haste makes waste. When mTORC1 activity was high and cells synthesized polypeptides rapidly, the stability of a newly synthesized fluorescent reporter protein was diminished. Inhibition of mTORC1, on the other hand, slowed protein synthesis through effects on translation initiation and elongation, and improved the fidelity of the process. Slower translation may allow more time for correct tRNA pairing or for cotranslational processes that promote proper folding and may thus result in fewer misincorporated amino acids or misfolded proteins, either of which would tend to reduce protein stability. — LBR

Sci. Signal. 6, ra24 (2013).

CELL BIOLOGY

Monocytes on the Prowl

Monocytes, macrophages, and dendritic cells are a family of cells collectively referred to as the “mononuclear phagocyte system” (MPS) that mediates and regulates inflammation. Cells of the MPS scavenge dead cells and toxic molecules, and kill or contain infectious agents. They are a critical mediator of the inflammatory response, which controls proliferation of microorganisms but they can also damage host tissues. Carlin *et al.* examined the role of a subset of monocytes, the Ly6C^{low} population, in mice. Ly6C^{low} monocytes, which continuously patrol the endothelium of capillaries, were shown to remove cellular debris and microparticles. In kidney tissues that bore the hallmarks of viral infection or cell death, the Ly6C^{low} cells were retained by the endothelial cells within the capillaries and recruited neutrophils, which mediated necrosis of the endothelial cells, and



CREDITS (TOP TO BOTTOM): SASHA TRUBETSKOY/WIKIMEDIA COMMONS; ADAPTED FROM L. CARLIN ET AL., *CELL* 153, 362 (2013)

then the monocytes cleaned up cellular remnants. Thus, these monocytes appear to serve as "intravascular housekeepers," orchestrating and cleaning up the damage caused by neutrophil-dependent necrosis of endothelial cells lining the capillaries. — SMH

Cell **153**, 362 (2013).

BIOMEDICINE

A Delicate Balance in Fragile X

Endocannabinoids are lipids that modulate cognition, anxiety, mood, and pain sensation—all behaviors that are deficient in patients with fragile X syndrome (FXS). FXS is a genetic disorder in which fragile X mental retardation protein (FMRP), an RNA-binding protein that controls protein synthesis, is not expressed in neurons. Treatments for the condition are focused on alleviating the symptoms. Busquets-García *et al.* suggest that targeting the endocannabinoid system could be a new therapeutic approach for FXS. When rimonabant, a drug that blocks endocannabinoid receptor CB1R, was injected into the hippocampus of FMRP-deficient mice (a good model for FXS), the animals showed improved memory and sensitivity to pain. In pyramidal neurons, activation of the receptor mGluR5 by excitatory glutaminergic neurons triggers the mTor signaling pathway to control gene expression and synaptic plasticity. Activation of this pathway is elevated in FXS, and rimonabant normalized the phosphorylation of pathway components Akt and p70S6K in the FXS mouse model. Genetic deletion of CB1R blunted the therapeutic effects of the drug. Furthermore, an antagonist of the CB2R endocannabinoid receptor specifically normalized anxiety-like behavior in the FXS mouse model. These observations suggest that modulating endocannabinoids in FXS patients could improve cognitive abilities and anxiety, among other symptoms. — LC

Nat. Med. **19**, 10.1038/nm.3127 (2013).

PHYSICS

A Very Dilute Superconductor

Whether a given material conducts electricity, or even does so without any resistance (as in a superconductor below its transition temperature), depends sensitively on the density of electrical carriers. This density can be manipulated in several ways, the most straightforward one being chemical doping. For a semiconductor (such as Si) to start superconducting, doping with an element (such as B) at a level of a few percent is normally

needed. Lin *et al.* found that in the bulk material SrTiO_3 , a tiny departure from the stoichiometric composition, achieved by removing 1 in 10^5 oxygen atoms, is sufficient to support superconductivity. Through thermoelectric measurements, they deduced that at this lowest carrier concentration the Fermi surface is almost spherical, with only a slight anisotropy, and that the Fermi temperature, a measure of the carrier concentration, is an order of magnitude lower than the Debye temperature, which reflects the lattice dynamics energy. This unusual hierarchy of scales, as well as the character of the Fermi surface, may present challenges to theoretical models of superconductivity in this compound. — JS

Phys. Rev. X **3**, 021002 (2013).

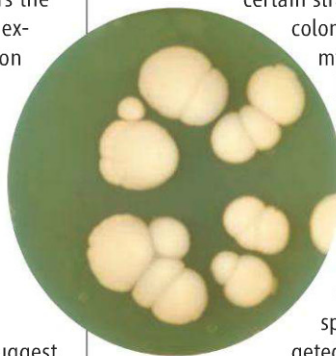
MEDICINE

Fugitive Fungi No More

Systemic fungal infections of the bloodstream or major organs, such as those caused by some species of *Candida*, can lead to fatalities, particularly in immunocompromised patients. The standard technique for detection requires culturing whole blood followed by morphological assessment, a process that can take several days. Neely *et al.* developed a whole-blood-compatible procedure that can detect certain strains down to one colony-forming unit per milliliter. After blood cell lysis and concentration of the *Candida* cells, polymerase chain reaction was used to selectively amplify the *Candida* DNA. This DNA was then captured with specific probes targeted toward one of five *Candida* species, which would

then agglomerate, giving clusters that could be detected with T2 magnetic resonance, using a custom-made bedside detector. For spiked samples, detection could be achieved within 3 hours, in contrast to the 1 to 2 days needed for the *C. krusei* and *C. albicans* species, respectively. Testing was also done on fully blinded specimens from patients who showed symptoms of septicemia, with excellent identification of both the *Candida*-positive and -negative cases. Three patients were also tracked in time, and in contrast to culture methods, which require viable fungal cells, the method could also detect nonviable cells after the treatment of patients with antifungal agents. — MSL

Sci. Transl. Med. **5**, 182ra54 (2013).



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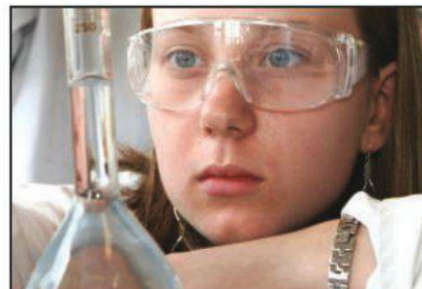


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Focus on Careers
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Science in Wales

The southwestern corner of the United Kingdom, with its population of just 3 million people, is making a bold bid for scientific greatness. In 2012, the government of Wales announced an agenda for scientific growth. The central program is built around recruiting research stars: high profile scientists who will move their laboratories to Wales and become part of national research networks. The program is funded with £50 million—nearly \$80 million. The Welsh government also wants to support science education, collaboration, and technology transfer, and quite simply, to be better at promoting Welsh science. Researchers are spreading the word about Wales as it launches an ambitious scientific plan.



See the full story on page 501.

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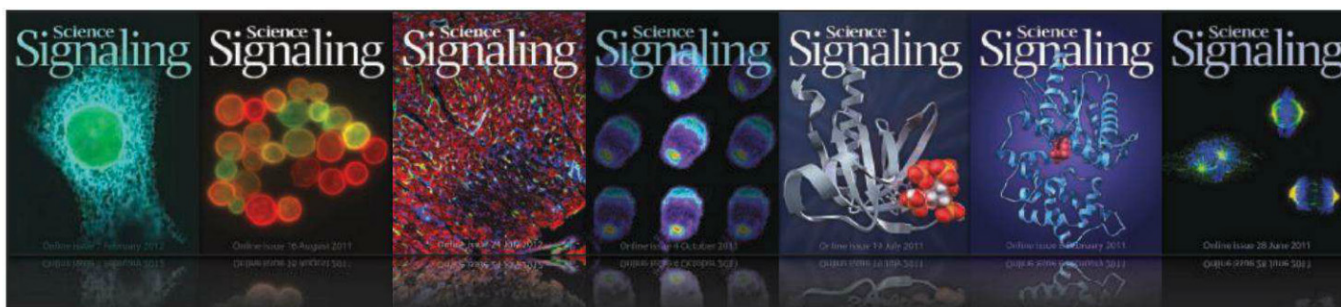
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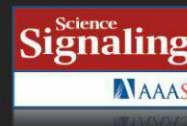
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AROUND THE WORLD



Swansea, U.K. 1

Measles Outbreak

Public health officials in South Wales are sending vaccination teams from school to school in an effort to contain a measles outbreak that could become the largest in the United Kingdom since the combination MMR vaccine (which also protects against mumps and rubella) was introduced in 1988. According to Public Health Wales, more than 800 people have fallen ill and 80 have been treated in hospitals since the epidemic, centered in the city of Swansea, began late last year. The body of a young man who died in the city last week while suffering from measles is also being examined to determine whether the virus caused his death.

Measles cases have increased in many European countries in recent years. In 2012, the Health Protection Agency counted 2016 measles cases in England and Wales, the highest number since 1994. The virus is highly contagious, and scientists estimate that 95% of people need to be vaccinated to protect against an outbreak. That coverage is rarely achieved in Europe, largely because many parents overestimate the risk of side effects of the vaccine and underestimate the risk that the disease poses.

Ya'an, China 2

Longmenshan Fault Ruptures Again

The dragon woke again, and this time scientists weren't caught off guard. On 20 April, a magnitude-6.6 earthquake struck 13 kilometers below the surface of Ya'an, a city of 1.5 million in western China's Sichuan Province. As *Science* went to press, Chinese authorities were reporting that more than 200 people lost their lives in collapsed

buildings and quake-induced landslides.

The temblor struck about 130 kilometers southwest of the epicenter of the 2008 Wenchuan earthquake, a magnitude-8.0 event that killed more than 80,000 people. Five years ago, the northern segment of the Longmenshan fault ruptured; last weekend, it was the southern segment's turn. Before Wenchuan, few geophysicists paid attention to the fault, which "has a relatively low slip-rate" and hadn't uncorked a big earthquake for



Firefighters search for survivors on 21 April.

centuries, says Mian Liu, a geophysicist at the University of Missouri, Columbia. But in the past 5 years, he says, researchers have been studying the fault extensively, and they detected stress building up along the southern segment. Where the next big Sichuan earthquake will strike isn't clear—but, Liu says, odds are on the Xianshuihe fault, west of Longmenshan.

Milan, Italy 3

Protestors Steal Animals, Destroy Research

Five people chained to the doors and valuable research mice wandering around free: That was the scene that greeted scientists with the University of Milan's Department of Medical Biotechnology and Translational Medicine on 20 April. During a national pro-

test against vivisection that drew 400 demonstrators to the university, five members of the pro-animal movement *Fermare Green Hill* (Stop Green Hill) illegally entered the department's building, also home of the Institute of Neuroscience of the National Research Council (CNR).

After hours of negotiations, the researchers were able to convince the intruders not to free most of the facility's animals, which are mainly used as models for neurodegenerative diseases. However, the intruders did take about a hundred transgenic mice and one rabbit and destroyed the identification cards on all the animal cages.

The following day, a group of university scientists openly protested, for the first time in Italy, against pro-animal ideology, and the University of Milan sued Stop Green Hill. "Our animal models are treated according to the European legislation. These people ruined years of research funded by national and European agencies" said Cecilia Gotti, a CNR researcher in Milan.

Vancouver, Canada 4

Ocean Fertilization Experiment Still Making Waves

Questions are still spinning around the legality of a geoengineering experiment conducted last summer in international waters. Last week, a native Canadian tribe filed a legal motion in provincial court in British Columbia to challenge a search warrant of its offices that was executed by Environment Canada in March. The search was part of an ongoing government investigation into the Haida Salmon Restoration Corp.'s controversial experiment, which involved dumping 100 tons of iron dust into an eddy 321 kilometers west of the Haida Gwaii islands to encourage the growth of algae and thereby increase fish stocks (<http://scim.ag/Haidafert>).

Environment Canada says that two international treaties on ocean dumping to which Canada is party prohibit the practice unless it can be deemed "legitimate scientific research." An attorney for Haida, which is affiliated with the Old Masset Village, a First Nation group in British Columbia, says that those treaties are nonbinding and that only domestic Canadian legislation can actually bar ocean fertilization. If the motion to challenge the search warrant is successful, the confiscated materials, including scientific records and files, will be returned. No arrests have been made or charges filed.

CREDIT: CDSB - IMAGINECHINA/AP PHOTO



How Low Can They Go?

The Great Lakes at the U.S.-Canadian border, with Asia's Lake Baikal and Africa's Lake Tanganyika, make up half the volume of the world's surface fresh water supply. This week, the National Oceanic and Atmospheric Administration (NOAA) reported that in January lakes Huron and Michigan dipped to their lowest point since monitoring began in 1860. The record continues almost 15 years of low water that began in 1998, a dry year with unusually warm water, extensive evaporation, and little precipitation and runoff flowing into the lakes. From 1998 through 2000, lake water levels dropped by about a meter, says NOAA hydrologist Andrew Gronewold, who is based in Ann Arbor, Michigan. Fish are cut off from the wetlands and rivers they need for reproduction, and giant commercial ships struggle to get through the channels that connect the lakes. NOAA and the U.S. Army Corps of Engineers predict lake levels will rise this summer, but not by much.

NEWSMAKERS

Take Two: Research on Aspirin and Heart Disease Earns Prize

A pair of pharmacologists has won the prestigious Grand Prix Scientifique, given annually by the Institut de France for work in cardiovascular health. **Garret FitzGerald** of the University of Pennsylvania and **Carlo Patrono** of the Catholic University in Rome

will share just over \$650,000 (€500,000) for disentangling how low-dose aspirin staves off heart disease.

"We competed, we collaborated, but we always remained friends," says FitzGerald of his relationship with Patrono.

Their work, performed mostly in the 1980s and early 1990s, came after years of clinical trials mysteriously failed to show low-dose aspirin helping the

heart. "People were saying, 'We know in a test-tube aspirin inhibits platelet formation,'" reducing clots, FitzGerald says. "'Why doesn't it in people?'"



FitzGerald



Patrono

Often racing each other, Patrono and FitzGerald sought to bridge the gap between test tube and human being. They elucidated how aspirin acts on platelets and identified both the ideal drug dose and who would benefit most: people with unstable angina, who experience a proliferation of platelets, leading to blood clots and heart attacks.

France Loses Molecular Biology Pioneer

French geneticist, World War II hero, and science writer **François Jacob** died on 19 April at the age of 92. Jacob was one of the fathers of molecular biology. Initially trained as a medical doctor and surgeon, he was severely wounded while serving as a medical officer for the Free French Forces. He began his research career in 1950 at the Institut Pasteur, when he began investigating how genes control enzyme synthesis. For his development of the "operon model," in which structural and regulatory genes coding for related



Jacob receiving his Nobel Prize.

proteins are arranged in units called operons, Jacob won the Nobel Prize in physiology or medicine in 1965, together with colleagues André Lwoff and Jacques Monod.

The operon model "had a major impact on biology as a whole," because it pooled biochemistry and genetics, and also "answered a question that geneticists had been asking for more than thirty years," said biologist and science historian Michel Morange, a former student of François Jacob, in an obituary released by the Institut Pasteur. Jacob received the highest French military decoration for World War II and was a former Chancellor of the Order of the Liberation.

FINDINGS

Radioactive Microbes Nuke Tumor Cells

Pancreatic cancer is grimly resistant to treatment, mainly because of the disease's ability to metastasize; only about 4% of patients survive for 5 years. But by delivering radiation directly to the cancer cells via genetically modified bacteria, researchers have hit upon a novel way to halt the disease's spread, they report this week in the *Proceedings of the National Academy of Sciences*.

The rod-shaped bacterium *Listeria monocytogenes* burrows into cells, causing severe illness such as meningitis. Because it can penetrate immune cells, some researchers use weakened *Listeria* with bits of tumor DNA attached to teach the body's immune system to recognize and destroy cancer cells. In 2009, immunobiologist Claudia Gravekamp, now of the Albert Einstein College of Medicine in New York City, found that *Listeria* not only spurred the immune system to attack the cancer cells, but also killed them directly.

Building on that work, she and her colleagues tagged modified *Listeria* with a radioactive isotope and injected it into mice carrying a highly metastatic form of pancreatic cancer. The radioactive bacteria therapy, they found, shrank the rodents' primary tumors by 64% while sparing healthy tissue; it also blasted cancer cells that had spread throughout the animals, reducing the number of metastatic cells by up to 90%.

Science LIVE

Join us on Thursday, 2 May, at 3 p.m. EDT for a live chat on **new exoplanet discoveries**. How close are we to finding a mirror Earth? <http://scim.ag/science-live>



Too close for comfort. Chickens, such as these at a Nanjing market, may transmit H7N9 to people.

INFLUENZA

Despite Large Research Effort, H7N9 Continues to Baffle

SHANGHAI, CHINA—If the influenza virus known as H7N9 had a bumper sticker, it would read, “HUGE MYSTERY.” Nearly a month after the first reported cases of people infected with the bird flu virus, many puzzles remain about how it made the jump to humans and adapted to us, how to prevent transmission, and how frequently an infection causes disease.

Public health experts have fitful nights about the virus touching off a pandemic. But even if that nightmare doesn’t come true, H7N9 may be here to stay, says Les Sims, an animal disease consultant in Palm Grove, Australia. “The fact that we have the virus in markets in three [Chinese] provinces and beyond tells me it would be a very difficult job to eliminate and eradicate the virus,” he says.

As *Science* went to press, the number of laboratory-confirmed human infections had risen to 108, with 22 fatalities. Around 10 international specialists arrived in Beijing last week to consult with the Chinese government; the group includes Keiji Fukuda, the top official for influenza at the World Health Organization (WHO).

A study published in *Eurosurveillance* on 18 April suggests that H7N9 has spread widely in domestic birds in China, under the radar, before passing to humans. The report’s authors—including Shu Yuelong, the director of the Chinese National Influenza Center in Beijing, and Marion Koopmans and Ron Fouchier of Erasmus MC in Rotterdam, the Netherlands—compared

the diversity of genetic sequences in H7N9 isolates to the genetic changes documented during outbreaks of two other intensely studied H7 viruses: H7N1, which emerged in Italy in 1999, and H7N7, which swept the Netherlands in 2003. The number of mutations in different H7N9 isolates, which came from several locations in eastern China and were found in people at roughly the same time, led the researchers to conclude that the changes likely occurred in birds.

Now that the virus has been discovered, it has become an acid test for China’s animal health infrastructure. Since the 2003 SARS episode and the near-simultaneous outbreak of H5N1, another bird flu strain, the country has focused on improving veterinary epidemiology, aided by training programs from the U.N. Food and Agriculture Organization (FAO). So far, “China is doing a fantastic job in reacting to this crisis,” says Vincent Martin, head of animal health for FAO’s Rome-based Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases.

A plan issued on 9 April by China’s Ministry of Agriculture—which did not respond to faxed questions—calls for collecting samples from every poultry market and slaughterhouse in provinces with confirmed human cases, along with more limited testing in all other provinces. Pig slaughterhouses are being targeted as well. It’s a “logical” strategy, Sims says.

And it’s being implemented on a massive

scale. China’s National Avian Influenza Reference Laboratory in Harbin has collected more than 84,000 samples, 47,801 of which have been tested, says the lab’s director, Chen Hualan. Only 39 were positive. Chen says that 19,978, or roughly 40%, of the processed samples involved serological tests, which look for antibodies that remain in the blood even after an animal clears the virus. Such tests are “crucial”, Sims says, because they detect past infections. The remaining tests were on cloacal and tracheal swabs, which capture the virus if taken only within a 2- to 3-week window when infected birds shed the virus in feces and saliva.

Wild birds may play a role in the virus’s spread as well. The H7N9 variants now in circulation most likely evolved by combining genes from viruses found in Beijing bramblings, Zhejiang ducks and Korean wild birds (*Science*, 12 April, p. 129). Over the last few weeks, researchers have collected 800 fecal samples from wild birds in wetlands in southeast China; they are now testing them, says Lei Fu-Min of the Institute of Zoology of the Chinese Academy of Sciences in Beijing. But so far, H7N9 has not been found in the wild save for a single infected pigeon.

One farmed pigeon also has tested positive, but all other infected birds—mainly chickens and ducks—were found for sale at marketplaces. Sims says he expects to find more infected birds at farms. “If you have the virus in a market,” he says, “it’s impossible to not have the virus somewhere on farms.”

Chinese authorities have already culled more than 100,000 birds and have closed many of the live animal markets in affected areas. At a press conference last week, Michael O’Leary, the head of WHO’s Beijing office, said that limited culling makes sense in places that have known infections but widespread culling—as was done with H7N7 and H7N1—does not. “I think in most situations it’s premature to do that now,” he said. That measure would be advisable, he said, if studies later show that the virus is easily transmitted in poultry.

If culling becomes the method of choice to control the disease and protect human health, it will be critical to offer fair reimbursement to farmers. Otherwise, “some farmers may hide their birds to prevent them from being killed,” says Chen Huanchen, a veterinary scientist at Huazhong Agricultural University in Wuhan. Current compensation poli-

CREDIT: YANG BO/COLOR CHINA PHOTO/AP IMAGES

cies vary by locality; Shanghai announced before it began culling on 6 April that farmers would be compensated for at least 50% of their losses, but many other areas are adhering to a 2005 government standard of \$1.61 per bird, which is widely seen as too low.

Meanwhile, surveillance has turned up scant evidence of transmission between people. One case of possible transmission from father to son has received extensive media attention, but it's possible they both had contact with the same bird, said O'Leary. Contacts of confirmed cases are typically tested for the virus or antibodies to it only if they develop symptoms, says Shu, thus mild cases might be missed. In addition to making it difficult to detect instances of human-to-human transmission, subclinical infections in people obscure bird-to-human spread.

On the other hand, there has been no direct contact with poultry in more than half the reported cases, O'Leary said. That suggests an unknown route of transmission—although O'Leary noted that “it is often difficult to determine all the exposures that the person might have, particularly when they



Deadly serious. H7N9 has caused severe disease in more than 100 people.

are thinking back a bit in time.”

To better assess H7N9 spread and disease severity in people, Shu and his co-authors suggest monitoring for conjunctivitis, or “pink eye,” the most common symptom found in the 89 humans who became infected during the H7N7 outbreak in the Netherlands. They also advocate looking for H7N9 antibodies in sera of people exposed to infected humans or birds. This might pick up asymptomatic cases as well as people who cleared infections, helping to clarify how the virus spreads. If

many such “silent” cases were found, it also would mean that the 20% fatality rate seen so far exaggerates the virus's true threat. “We are going to collect sera samples from high risk groups such as poultry workers to assess the risk of human infection,” Shu wrote *Science* in an e-mail.

However, detecting H7N9 antibodies in human sera is difficult. Decades-old studies have shown that the most commonly used test for influenza antibodies—the hemagglutination inhibition assay—doesn't work well for gauging mammalian immune response to avian viruses. That's why many groups rely on microneutralization assays, a more cumbersome and time-consuming test for avian viruses in humans that requires growing large amounts of the pathogen, says Gregory Gray, an epidemiologist at the University of Florida in Gainesville. “We need better assays,” Gray says. It's only one of the many urgent challenges posed by a virus that 1 month ago was unknown.

—MARA HVISTENDAHL, DENNIS NORMILE, AND JON COHEN

With reporting by Della Fu in Shanghai.

U.S. IMMIGRATION REFORM

More High-Tech Visas, More STEM Education Funds

Valentina Waters always got good grades in math and science. But college was out of the question: As one of 11 siblings, she needed to work after graduating from high school to help support her family.

More than a decade later, however, Waters is studying chemical engineering at Saddleback College in southern California and hopes to carve out a career in the pharmaceutical industry. Although born in Russia, she became a permanent U.S. resident last year. And her dream is being realized with funds from an unexpected source: fees that the U.S. government charges high-tech companies and universities that want to employ a foreign scientist or engineer. It's a pot of cash that could soon grow significantly.

Last week, a bipartisan group of U.S. senators unveiled a sweeping proposal to overhaul the nation's immigration policies. The bill would create new funding streams aimed at strengthening STEM (science, technol-

ogy, engineering, and mathematics) education and training programs for U.S. students like Waters and enrich existing mechanisms. It's based on the idea that companies that hire foreign-born STEM workers should also help expand the homegrown STEM workforce. The proposal would also make it much easier

“just completely broken,” Senator Marco Rubio (R-FL), one of the “Gang of Eight” senators behind the proposal, told reporters. The 844-page bill (S. 744) aims to fix it by, among other things, creating a path to citizenship for an estimated 11 million undocumented immigrants already in the United States and beefing up border security. It would also, Rubio said, “prioritize recruitment of immigrant entrepreneurs, innovators, investors, [and] skilled workers.” However, the plan faces uncertain prospects in Congress.

The bill fundamentally alters how some 1 million people a year win the legal right to permanently live and work in the United States. Under current policies, nearly three-quarters receive their permits, or “green cards,” as a result of family ties to people already in the country. Fewer than 15% receive green cards based on their education or skills. The proposal would move to a nearly 50-50 balance in coming decades



STEM at work. Saddleback College engineering students visit California State University, Fullerton, in program funded by visa fees.

for foreign-born scientists and engineers to live and work in the United States, especially if they earn an advanced STEM degree from an American university.

The existing U.S. immigration system is

by creating more and easier paths to entry for foreigners with technical skills.

One change would increase the proportion of so-called “employment” visas reserved for more educated applicants. They could receive 40% of the 140,000 visas issued annually, up from 28.6% now. Foreign-born students who earn a master’s degree or higher in a STEM field from a U.S. institution in the preceding 5 years would automatically qualify—an approach popularly known as “stapling a green card to their diploma.” That proposal is a “positive step ... although nothing is ever solved in one fell swoop,” says Michael Crow, president of Arizona State University in Tempe.

Crow is one of many academic and high-tech officials who have found a lot to like in the proposal. They support a provision that would create 120,000 new “merit-based” visas to be awarded via a scoring system; 50% would be reserved for skilled workers, who can earn points for advanced degrees or starting businesses. The number of merit visas could grow to 250,000 over time. An additional 10,000 green cards would go to entrepreneurs who raise \$500,000 in capital or generate \$750,000 in U.S. sales and employ at least five people. And, echoing existing law, an unlimited number of visas would be available to “extraordinary” or “outstanding” researchers, such as Nobel laureates, as well as those holding doctoral degrees in any field.

The proposal also expands the number of temporary visas available to technical workers—a priority for industry. The number of H1-B visas, which last up to 6 years, would grow from 65,000 to 110,000 and could go as high as 180,000 depending on economic conditions. The legislation also adds 5000 to the 20,000 additional H1-Bs now available to foreign students who earn advanced degrees in STEM fields from U.S. colleges and universities (but not in the life sciences, where supply exceeds demand). The proposal also specifies that some of the fees paid by visa and green card applicants would be used to strengthen STEM education and training for U.S. students.

Making that link is good public policy, says Pruthikrai (Winn) Mahatanankoon, a professor of information systems at Illinois State University in Normal. He’s living proof: A native of Thailand, he was able to take and

remain at his academic job after earning a Ph.D. from Claremont Graduate University in northern California thanks to an H-1B visa and, later, a green card. On 17 May he will become a naturalized U.S. citizen.

Mahatanankoon is already giving back to his adopted country. In 2010, he received a \$600,000 grant from the National Science Foundation (NSF) to run a scholarship program for low-income students in computer science that is funded by H-1B visa fees. He didn’t know the source of the funding for the so-called S-STEM program when he applied.



Building a career. Valentina Waters gets hands-on experience during a field day at Fullerton.

But he was thrilled to find out. “The circle has come around,” he says. “This is exactly what the United States should be doing to help create more domestic STEM workers.”

Since 1998, a portion of the \$1500 fee paid by H-1B visa petitioners has generated more than \$1 billion for STEM education and training programs at the Department of Labor (DOL) and NSF. Those efforts, which include helping students of modest means and those from groups traditionally underrepresented in STEM fields, have been a godsend for students like Waters, who might otherwise never have pursued a STEM career. Now, she’s one of 35 students in a program called Bridge to Engineering that is funded with a DOL grant.

“If I had just gone back to school I wouldn’t have gotten the chance to learn so much about opportunities in the field, take field trips and build a network,” says Waters, a 28-year-old expectant mother with a husband in the military. This summer Waters will intern at NASA’s Jet Propulsion Laboratory in nearby Pasadena courtesy of the DOL program. She also hopes to receive an NSF S-STEM scholarship that would pay for the rest of her associate degree.

Now, DOL gets 55% of the H1-B fees for worker retraining programs and other uses, while NSF receives 40%. That generates about \$100 million a year for two NSF programs: S-STEM gets about \$75 million, and the rest is channeled into ITEST (Innovative Technology Experiences for Students and Teachers), which supports a range of pre-college STEM activities involving university researchers and local school districts.

The Gang of Eight bill would add a third NSF program supported by H-1B fees and designed to boost STEM programs at colleges and universities with large minority populations. The legislation would also create a second pot of money for STEM education, drawn from a new \$500 fee for green cards. NSF would get 85% of that second pot, to be divided among the three programs.

For scientists like Amy Wendt, a plasma physicist at the University of Wisconsin, Madison, those changes would mean more opportunities to grow the STEM pipeline. She and her Wisconsin colleagues received \$1 million in 2010 from ITEST to develop socially relevant course units in engineering for middle school students.

“The research shows that some students may be attracted to humanitarian opportunities in engineering,” says Wendt, who says that she’s been disappointed that the percentage of women in electrical and computer engineering hasn’t grown much since she entered the field 3 decades ago. “A girl who’s not into a robotic competition might be interested in solving an environmental problem. But we as a community haven’t done a very good job of highlighting the altruistic aspects of our discipline.”

Exactly how much money the new fees would generate isn’t clear, because of uncertainty about how many new visas would be awarded under the Senate legislation. Estimates range from \$50 million to several hundred million a year.

Whatever the amount, Wendt and Mahatanankoon say they are pleased that immigration fees are being tapped to encourage more U.S. students to study science and engineering. “If our program is successful, I plan to apply again,” Mahatanankoon says. “These students need all the help we can give them.”

—DAVID MALAKOFF AND JEFFREY MERVIS

CREDIT: KATLIN CHOI



ARCHAEOLOGY

Deep Dig Shows Maya Architecture Arose Independently of Olmec's

The origins of the Maya civilization remain one of archaeology's longest-running mysteries. The Maya continually renovated their imposing pyramids and plazas, burying the earliest architecture under thick layers of stone. So researchers have long struggled to answer a basic question: Did the Maya inherit much of their civilization from the Olmec people in southern Mexico, whose first major ceremonial center, San Lorenzo, arose around 1400 B.C.E.? Or did Maya civilization arise in a more complex way, through interactions with many societies in the region, and only a small helping hand from the Olmec?

A study published this week in *Science* (p. 467) on new data from the Maya city of Ceibal in Guatemala strongly suggests that one key element of Maya civilization—the arrangement of urban ceremonial space—owed little to the Olmec. In a major dig, a team led by archaeologist Takeshi Inomata of the University of Arizona, Tucson, discovered the remains of a ceremonial core, including formally arranged platforms and a plaza, dating to 1000 B.C.E. The platform arrangement is the oldest known standardized ceremonial compound in Mesoamerica—and it predates the first appearance of such architecture among the Olmec. Early Maya may have used the platforms as a stage for ritual performances, and they later transformed this architecture into the Maya lowlands' first plaza and pyramid complex—a hallmark of their later civilization.

The formal spatial plan reveals the Maya's early sophistication, says archaeologist Francisco Estrada-Belli of Tulane

University in New Orleans, Louisiana. The study “really opens the door to the idea that the Maya were not the recipients of cultural influence from [the Olmec], as has been suggested,” he says.

The Olmec lived in Mexico's Gulf Coast region, and carved massive human heads—likely portraits of rulers—from 20-tonne



blocks of basalt. The Maya took up sedentary living after the Olmec did, around 1000 B.C.E.—about when the Ceibal complex was built.

Inomata and his team were drawn to Ceibal by the findings of Harvard University archaeologists who discovered very early Maya ceramics there in the 1960s; they also found a later arrangement of ceremonial space that resembled layouts found at the Olmec capital, La Venta, and at early

From the bottom. Archaeologists uncovered the roots of Maya plazas and platforms at Ceibal.

sites in Mexico's Chiapas region (see map). To examine the origins of this spatial plan, Inomata's team opened major excavations at Ceibal in 2005.

Sinking 12-meter shafts and tunneling below pyramids, the team discovered Ceibal's earliest public architecture: a plaza containing ritual deposits of greenstone axes; an earthen platform that may have held an elite residence; and parts of a characteristic architectural arrangement called an E-group assemblage—a square platform in the west and a long platform aligned north-south. Radiocarbon dating showed that this ceremonial core was built during a transitional period, between the fall of San Lorenzo around 1150 B.C.E., and the rise of La Venta, around 800 B.C.E.

Because San Lorenzo lacks large mounds and pyramids and the later La Venta has a pyramid and plaza complex, Inomata suggests that this kind of ceremonial compound emerged during a time of social ferment when the Maya and many other Mesoamerican groups, including those in the Chiapas area and along the Pacific coast, were communicating and experimenting with new ideas and social orders. “I’m not saying that Ceibal was the origin [of the new architecture], but it was part of a new movement in a broader area,” he says.

The dating evidence is sound and “overwhelming,” says archaeologist Michael Love at California State University, Northridge. Unpublished results from other sites point in the same direction, he adds.

Not everyone is ready to abandon the model of the Olmec as the major cultural source of the early Maya, however. The new research strongly suggests that the idea of E-groups did not come from La Venta, agrees archaeologist John Clark of Brigham Young University in Provo, Utah. However, such formally arranged ceremonial architecture could still be discovered at San Lorenzo, he says.

Inomata agrees that more excavation is needed but says the finds at Ceibal shed important new light on the early days of Maya civilization. “When some people think about the emergence of civilization, they think of the development of writing and kingship, but this form of spatial organization, and the social organization it implies, probably plays a really critical part,” he says.

—HEATHER PRINGLE

Heather Pringle is a contributing editor at *Archaeology* magazine.

Dark-Matter Mystery Nears Its Moment of Truth

As announcements go, it was a bit like texting your parents that you're getting married. At a meeting last week, physicists working with an ultrasensitive particle detector deep underground reported three blips that could be particles of dark matter, the mysterious stuff whose gravity binds the galaxies, bumping into atomic nuclei. However, the leaders of the on-going Cryogenic Dark Matter Search (CDMS) issued no press release and stressed that three "events" are too few to claim a discovery.

Nevertheless, other physicists are taking note for two reasons. First, they say, the three events are cleaner and more persuasive than earlier ones CDMS recorded. Second, if CDMS has spotted dark matter, then the beast they've glimpsed—a lightweight version of a so-called weakly interacting massive particle (WIMP)—should show up in other experiments in a year or so. "If this is real, we should know soon," says Neal Weiner, a theorist at New York University in New York City.

CDMS researchers had reason to be cautious. In December 2009, as rumors swirled, they presented two other possible WIMP events. Even though a press release that they issued claimed no discovery, others rolled their eyes. CDMS researchers used certain criteria, or "cuts," to sift signal events from "background" events caused by ordinary particles. If the team tightened its cuts a tad, the two events went away, leading some physicists to criticize CDMS researchers for calling them possible signal events at all. "We felt a bit burned by the experience," says Blas Cabrera, a CDMS member from Stanford University in Palo Alto, California.

This time CDMS researchers soft-pedaled the data. Lurking 713 meters down in the idled Soudan mine in northern Minnesota, CDMS comprises disks of germanium or silicon cooled almost to absolute zero. To look for a nucleus recoiling from a collision with a WIMP, researchers monitor each disk for a pulse of electricity teamed with a pulse of heat. The previous events appeared in germanium data in a version of the experiment known as CDMS-II. (Researchers are now running SuperCDMS.) The new events come from silicon data that CDMS-II collected

from July 2007 to September 2008 and were presented on 13 April at the meeting of the American Physical Society in Denver. "They look robust, they look healthy," says Juan Collar, a physicist from the University of Chicago in Illinois, who does not work on CDMS.

Still, the events should be viewed with caution, physicists say. To interpret their data, CDMS researchers must estimate the number of background events that on average they would expect to find in such a data set, which they calculate to be 0.4. That number and

of events that could be low-mass WIMPs. In 2011, researchers working with the Cryogenic Rare Event Search with Superconducting Thermometers (CRESST) in Italy's subterranean Gran Sasso National Laboratory spotted similar signs.

However, an experiment known as The XENON Dark Matter Project in Gran Sasso claimed to have ruled out the CoGeNT and CRESST signals, and a food fight broke out over whose measurements were reliable (*Science*, 3 June 2011, p. 1144). XENON used a detector filled with liquid xenon, an element whose heavy nucleus makes it less sensitive to lightweight WIMPs. So some researchers argued that the XENON team overstated the detector's sensitivity. Conversely, reanalysis of the CoGeNT results suggested that they were mostly background events.

If light WIMPs exist, then several other experiments should soon see them. For example, the Large Underground Xenon dark-matter experiment (LUX) has been assembled 1478 meters down in the Sanford Underground Research Facility in Lead, South Dakota, and should start taking data this year. With its mass of 350 kilograms of frigid liquid xenon, LUX should see thousands of light WIMPs, if they're there. "This is the year of the light WIMP or of its demise," Collar predicts.

The acid test for the entire notion of WIMPs may not be far behind. The idea emerged from a concept in particle physics called supersymmetry, which posits that every known particle has a more massive "superpartner."

The lightest of these would be a stable uncharged particle that hardly interacts with normal matter—just the ticket for dark matter. Supersymmetry generally predicts that the particles should be hundreds of times as massive as a proton, and that's mainly the type of particle physicists have sought.

But it hasn't shown up yet. If it doesn't appear in the new tonne-scale detector XENON is building or similar detectors that researchers in the United States hope to build by 2016, then physicists may have to accept that even if WIMPs exist, they're undetectable. "It's going to a critical juncture," says Lian-Tao Wang, a theorist at the University of Chicago. "We are living interesting times."

—ADRIAN CHO



Tantalizing. Physicists working on the Cryogenic Dark Matter Search have spotted three potential signals of dark-matter particles.

other details imply that there's a 0.2% chance the three events are due to background alone, they say. But if the researchers have underestimated the background a little, the significance of the result falls a lot, says Richard Gaitskell of Brown University. "It's like brain surgery," he says, "one little slip and it changes the outcome entirely."

CDMS's results suggest that WIMPs are about eight times as massive as the proton—less than theories had generally forecast—and they roughly jibe with other hints of lightweight WIMPs. In 2010, Collar and colleagues working with an experiment called the Coherent Germanium Neutrino Technology (CoGeNT) in Soudan reported an excess

LATIN AMERICA

In Quest for Synchrotron, Brazil Tests Homespun Ingenuity a Second Time

Nearly 30 years ago, Ricardo Rodrigues helped build a synchrotron from scratch. That was no mean feat, as the young engineer at the University of São Paulo and his Brazilian colleagues were hampered by unstable funding and a shortage of trained personnel. Switched on in Campinas, Brazil, in 1997, the machine, known as UVX, was more artisanal than state-of-the-art. But Rodrigues, now head of engineering at the Brazilian Synchrotron Light Laboratory (LNLS), which houses UVX, won't have to rest on that laurel: He's getting a second chance to build his dream machine.

Early next month, LNLS scientists will break ground on Sirius, a synchrotron x-ray source whose energy and brightness will rival those of the best machines in the world. The \$330 million initiative is the latest sign that Brazil is "taking the lead" in Latin American science, says Galo Soler-Illia, a chemist at the University of Buenos Aires and a member of Argentina's National Atomic Energy Commission. While other countries in the region shy away from huge investments in science, "these guys [in Brazil] are just building it," says Soler-Illia, who served on LNLS's scientific committee in 2011 and calls Sirius a "bright star" for Latin America.

In many ways, UVX was a more daunting challenge. It had to be conjured from thin air. "When we started building [UVX], we had five people in the country that had used synchrotron radiation. I was one of them," says Rodrigues, who had spent time at DESY in Hamburg, Germany, while a postdoc at King's College London in the 1970s. Lacking the financial and scientific resources to recruit foreign researchers to work on UVX, Rodrigues hired mostly young Brazilian scientists and engineers looking for their first jobs out of university. "They had to learn by doing," he says. Skyrocketing inflation back then made it prohibitive to buy equipment from overseas, and local industry lacked the technical skills to manufacture items such as

magnets and vacuum lines. So Rodrigues and his team constructed the machine by hand.

Their labors paid off. Scientists have traveled from as far away as Germany and Norway to use Latin America's only synchrotron. But most of UVX's 1500 or so users a year hail from Brazil. Their primary research interests are in fields that have long benefitted from synchrotrons, such as materials science, condensed matter physics, and structural biology. Powerful oil companies like Petrobras and Braskem also rely on UVX for R&D.

Even as its local user commu-



Past triumph. Ricardo Rodrigues, a mastermind of UVX (pictured), says that Sirius will be a bigger and brighter x-ray source and therefore much more useful.

nity grew, UVX, a 1.4-GeV machine, diminished in scientific relevance. "It's a second generation machine," explains LNLS Director José Roque, a physicist. "It has relatively low energy, a large emittance, and thus a low brightness," making it impossible to keep up with modern third-generation machines in areas such as 3D nanoscale imaging. As a result, many Brazilian scientists who cut their teeth on UVX now go abroad to push their research forward—the type of brain drain that UVX was supposed to help staunch.

LNLS realized that the generation of Brazilian scientists who came of age at UVX had outgrown the machine. In 2009, the lab asked Rodrigues, then running his own struggling technology company, to return and build a bigger and better synchrotron. Brazil's science ministry has come up with the lion's share of funding; Roque hopes to secure additional support from other agencies and companies like Petrobras.

With a diameter of 165 meters, Sirius will

be five-and-a-half times the size of UVX. And its 3 GeV energy will put it on par with machines like Diamond Light Source in the United Kingdom and SOLEIL in France. With respect to emittance, Sirius will outshine its rivals. Low emittance means a narrow and bright x-ray beam that is more useful for imaging the nanostructure of materials ranging from oil to bacteria to fossils. Sirius will boast one of the lowest emittances in the world: 0.28 nanometer-radians. For scientists accustomed to UVX's 100 nanorads, it will be like "going from an old TV to an HDTV set," says LNLS Scientific Director Harry Westfahl. "All of a sudden you see everything more bright[ly]."



After finishing a postdoc at the University of Illinois, Urbana-Champaign, Westfahl returned to his homeland in 2001 to head LNLS's theory group. He's now overseeing construction of Sirius's first 13 beamlines. (Ultimately, the machine will have room for about 40.) He hopes that their sophisticated end stations—some of which are under construction for UVX—will entice more overseas Brazilian scientists to come home. Scientists from across Latin America are expected to flock to the new facility as well.

First, however, Rodrigues and his colleagues must summon their ingenuity and audacity a second time. The optics required for such a low-emittance machine are "on the edge of what is able to be done nowadays," Westfahl says. And the Sirius team must hire an army of scientists to meet its ambitious target of hosting its first users in 2017. The tasks ahead are "practically Olympic," Soler-Illia says. The LNLS team will rise to the challenge, predicts Chi-Chang Kao, director of the SLAC National Accelerator Laboratory in Menlo Park, California. "They've done it before," he says. "It's just a much bigger scale this time around."

As for UVX, Rodrigues feels attached to his firstborn. While "Sirius can do all [that UVX] does much better," he says, "it would be nice if we could keep [UVX] running somewhere else," perhaps by moving the storage ring to another country that wants to kick-start its own synchrotron science program. "I would be very happy with that."

—LIZZIE WADE

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INTELLECTUAL PROPERTY

In a Flurry of Metaphors, Justices Debate a Limit on Gene Patents

The U.S. Supreme Court took a heady plunge into molecular biology on 15 April as it tried to determine whether patents on the cancer-linked genes *BRCA1* and *BRCA2* are legitimate. The debate turns on whether DNA sequences patented by Myriad Genetics Inc. of Salt Lake City are human inventions, as Myriad claims. If not, the court could rule that they are “products of nature” and void the patents. A decision is expected before the end of June.

Challengers have been waging a legal campaign to topple Myriad’s 15-year-old patents since 2009. A group of researchers, clinicians, and legal advocates represented by the American Civil Liberties Union (ACLU) argues that patents on *BRCA* genes—the basis of Myriad’s commercial test for breast and ovarian cancer risk—lock up nature and impede research. In 2010, a New York federal court ruled that the patents are invalid; in 2011, the Court of Appeals for the Federal Circuit overruled that decision. The challengers then appealed to the Supreme Court. (Australia’s highest court last week also began considering whether to strike down *BRCA* patents owned by Myriad and the diagnostics company Genetic Technologies Ltd. of Melbourne.)

Judging by questions and answers in the U.S. proceedings last week, research findings could play an important part in the court’s decision. The Supreme Court veered into scientific territory after Justice Stephen Breyer cited a technical analysis submitted by mathematician-biologist Eric Lander, a leader of the Human Genome Project and founder of the Broad Institute in Cambridge, Massachusetts. Claiming to speak for neither side, Lander filed an amicus brief arguing that the recent lower-court decision in favor of Myriad is simply wrong. That earlier ruling accepted Myriad’s claim that “isolated” *BRCA* genes are laboratory products and that, unlike chromosomes, they do not occur in nature. Lander writes that this “is demonstrably incorrect.”

Lander, who co-chairs the President’s Council of Advisors on Science and Technology, writes in his brief that chromosomes are constantly breaking up in the body, yielding fragments of genomic DNA. Specifically, he points to studies published last year in *Nature* and *Science Translational Medicine* (a sister publication of *Science*) reporting that blood

from pregnant mothers contains enough isolated DNA fragments from the fetus to enable one to reconstruct the fetus’s entire genome. Data available online from Stanford University, according to Lander, show that “isolated fragments of fetal DNA in maternal blood cover the *BRCA1* and *BRCA2* genes.” He concludes, “isolated DNA fragments from the human genome,” including those claimed in Myriad’s patents, “are products of Nature, not the handiwork of humans.”

Breyer queried Myriad’s lawyer, Gregory Castanias, many times about Lander’s brief.



Who owns genes? Protesters gathered at the Supreme Court as it weighed the fate of *BRCA* gene patents.

“Have I misread what the scientists told us, or are you telling us the scientists are wrong?” Breyer asked. Castanias did not address the question directly. But he said that the DNA fragments that Lander described would not amount to “something that is new and useful and available to the public.”

For much of the session, the U.S. justices suggested different metaphors in an effort to fit the biology into a legal mold. They appeared to be looking for a way to distinguish between artificial DNA constructs (which might be patentable) and naturally occurring genes (which might not). Justices Samuel Alito and Ruth Bader Ginsburg com-

pared Myriad’s patented genes to a molecule taken from an imaginary tree deep in the Amazon. They suggested that the tree and its leaves might not be patentable, but a concentrate of sap used as medicine might be. At another point, the justices considered whether the *BRCA* genes might be like a baseball bat carved out of a tree. (The bat might be patentable but not the wood or the tree.) Justice Sonia Sotomayor compared the gene sequences to ingredients in a chocolate chip cookie. She said she could imagine a patent on a method of combining the ingredients, but, “I can’t imagine getting a patent simply on the basic items of salt, flour, and eggs.” The metaphors may not have been perfect, but Lander sees them as an attempt to express in lay language “a serious understanding” of the key issues. He says: “I was impressed by how the justices recognized that science is at the heart of the case.”

Some justices also expressed concern about the economic impact of doing away with human gene patents. Justice Elena Kagan asked the ACLU attorney Christopher Hansen: “Why shouldn’t we worry that Myriad or companies like it will just say, well, you know, we’re not going to do this work anymore?” Hansen responded: “There were other labs doing *BRCA* testing” before Myriad began to enforce its patents, and “Myriad shut all that testing down.” He added that the “whole point” of ACLU’s argument is that “when you lock up a product of nature, it prevents industry from innovating and making new discoveries.”

The Obama administration, in arguments presented by U.S. Solicitor General Donald Verrilli, proposed a compromise of sorts. Although the U.S. government has allowed gene patents in the past, Verrilli said, it now believes that “isolated” DNA of the kind in Myriad’s patents should not be patentable “because it is simply native DNA extracted from the body.” He said that patents should only be allowed on DNA sequences that are clearly an “artificial creation in the laboratory,” such as complementary DNA, or cDNA. Myriad’s lawyer, Castanias, said that virtually all the objections being made against the *BRCA* patents were considered in a thorough review 12 years ago by the U.S. Patent and Trademark Office—and rejected. He didn’t see the relevance of the new distinctions proposed by the government, but several justices seemed ready to adopt them.

—ELIOT MARSHALL

With reporting by Leigh Dayton.

Room for hope? Thanks to donations and preferential grain rations, conditions are improving at the Ryokpo rest home and other TB facilities.

Public Enemy Number One

North Korea has one of the highest TB rates outside sub-Saharan Africa and a burgeoning drug-resistance problem. A remarkable collaboration is hoping to turn the tide

UNPA, NORTH KOREA—This village in rugged hills 2 hours south of Pyongyang has had it rough. Last summer, a typhoon wiped out most of its corn crop, and a second windstorm ripped stone tiles from roofs. Clinging to a slope facing the settlement, the Unpa tuberculosis (TB) rest home—a tidy gray concrete building with a red roof—took a battering. None of the staff members or 46 patients was harmed. But the hurricane-force gales shattered windows of a ward under construction that will house patients infected with multidrug-resistant (MDR) TB and sent its steel roof sailing down the valley. Surveying the damage, Heidi Linton, executive director of Christian Friends of Korea (CFK), a humanitarian organization based in Black Mountain, North Carolina, jots down a list of materials that CFK intends to purchase for the MDR wing. Occasional booms punctuate the stillness: Distant artillery fire as North Korea prepares for what it sees as an inevitable military conflict with the South.

As war fever reached a frenzied pitch last month, Linton and six other Americans were in the countryside with North Korean scientists and physicians, joining forces against a common enemy: tuberculosis. TB has skyrocketed in the Democratic People's Republic of Korea (DPRK) in the past 20 years, according to the World Health Organization (WHO). Famines in the mid-1990s ignited the epidemic; chronic malnutrition ever since has added fuel to the fire. North Korea now has one of the highest TB incidences outside sub-Saharan Africa.

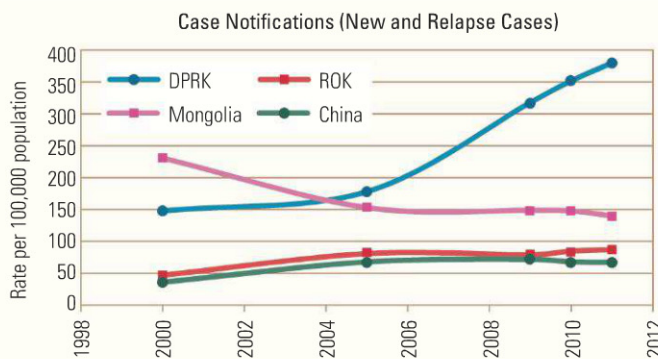
That makes it unusual. Nearly all other countries with high TB case rates also have high HIV/AIDS rates. HIV suppresses the immune system, allowing TB to flourish. In North Korea, “essentially there is no HIV,” says Gary Schoolnik, an infectious disease researcher and physician at Stanford University School of Medicine in Palo Alto, California. “That’s why,” he says, “the magnitude of the TB problem in

Laboratory (NTRL) in Pyongyang. The effort is a rare example of scientific cooperation between North Korea and the United States and, at a time of heightened tensions, one of DPRK’s few active links with the West. NTRL researchers can now diagnose TB cases that are resistant to first-line drug combinations, making it possible to spot patients who need more aggressive therapy. And the lab will soon add capacity to screen for extensively drug-resistant TB, known as XDR—the worst strains, some of which are close to impossible to treat. “This is a critical point in the development of their program, says Kathleen England, a Stanford microbiologist who is spending weeks at a time

at North Korea’s NTRL to help it win international accreditation, as early as 2015. Attaining that gold standard, says Schoolnik, who has been involved in the project since its inception, “would allow North Korea to join the global fight against TB.”

Last month, I joined Linton’s team on visits to four rural TB rest homes and two facilities in the capital: NTRL and the pediatric ward of the Pyongyang District #3 TB Prevention Hospital. (The DPRK government cleared my visit as a journalist.) The trip came

at a delicate time, as South Korea and the United States were conducting annual military exercises that included stealth bomber flights over South Korea. Angered by a fresh round of U.N. sanctions imposed last month over its third nuclear test, North Korea’s response to the drills was to sever hotlines, “scrap” the armistice ending the Korean War, suspend operations at the Kaesong industrial park—a joint venture with the South—and issue bellicose threats. Last month, state television aired interviews with North Koreans railing against U.S. imperialists and showed leader Kim Jong Un inspecting garrisons and planning military maneuvers. Buses and locomotives were festooned with camouflage netting, and a new propaganda mural in Pyongyang depicts a



Rough decade. North Korea is struggling to rein in its TB epidemic.

missile bearing down on Capitol Hill. “I’ve never seen things ratcheted up so high,” says Linton, who has made more than 40 trips to DPRK since 1998 on missions to renovate hospitals and oversee distribution of donated food and materials, mostly in the southwest.

North Korea may believe that its enemies are poised to strike. But TB, one of humanity’s most enduring scourges, is already running rampant across the isolated nation.

Empty bellies

Along the highway leading south from Pyongyang, work brigades are out in force. One group of villagers is tilling paddies in preparation for transplanting rice shoots later in the spring. Lines of red flags on wooden poles flutter in the breeze, a DPRK way to promote camaraderie. A brigade of women is working on railroad tracks, using their hands to even out gravel between the ties, their bicycles lying haphazardly along the elevated bed. In a field down the road, a line of red signboards, each emblazoned with a white Korean letter, declares “Comrade Kim Jong Un, live 10,000 years!”

At the Unpa TB rest home, Director Ko Gwang Sung invites us into a reception room. One wall shows a painted landscape of fractured granite peaks lapped by ocean waves: “Sea Kungang,” the coastal range of the Diamond Mountains in the southeast. Linton, who has been to Unpa nearly a dozen times, runs through a list of questions that she has prepared for each rest home. She takes the sanatorium’s vital signs, quizzing Ko on how many doctors, patients, and beds the sanatorium has; the number of patients with infectious pulmonary TB; and whether drug stocks are sufficient.

In much of the world, TB is in retreat. A 2012 WHO report notes that new TB cases have been falling for several years; since 1990, the mortality rate has decreased 41%. “[T]he world is on track to achieve the global target of a 50% reduction by 2015,” WHO states. North Korea is bucking that trend. In 1994, it reported fewer than 50 cases per 100,000 people—a third of the rate of South Korea. By 2011, North Korea’s TB incidence had climbed to 374 cases per 100,000 people—four times the rates in China and South Korea (see graph on facing page). Although there “certainly could have been significant underreporting” over the years, Schoolnik says, “I think the increase is real.” WHO estimates that among North Korea’s 24 million people, there are 91,200 active TB cases. That figure is a stab in the dark. “We are not able to get numbers from everywhere, so we assume the rate is higher,” England says. Officials in Pyongyang keep data close to the vest. “Data are not being shared,” England says.

Ko, like other Korean TB workers, says that recently improved case detection could explain the stubbornly high incidence. In southwestern DPRK, at least, “cases are leveling off,” says Ko, who witnessed how the epidemic began to unfold 2 decades ago. But by no means is there a consensus. “I’m not convinced it’s slowing down. We may have a ways to go before we hit the top of that curve,” Linton says.

One point of agreement is that the roots of the present crisis lie in an epic famine that North Koreans call the Arduous March. Between 1994 and 1998, hundreds of thousands of Koreans starved to death; hunger-weakened survivors were vulnerable to infections. TB rates “climbed significantly,” England says. The bug is everywhere: One out of every three people in the world has a latent TB infection that a healthy immune system usually keeps at bay. But in malnourished people, TB can break out of its cage and multiply in the lungs. A person with untreated pulmonary TB infects 10 to 20 people on average, Schoolnik says.

Online

sciencemag.org

Slides and podcast interview with Richard Stone (http://scim.ag/pod_6131).

Across North Korea, county governments oversee collective farms, gather up harvests, ship food to Pyongyang and to army outposts, and dole out what’s left to the rest of the population. Linton’s team has learned from its recent visits to rest homes that the government has set the grain ration for adult TB patients at 450 grams per day, handed out as corn or rice. The ration “is an improvement over previous years,” Linton says. “It tells me that the DPRK government may finally recognize that TB patients should be prioritized in the distribution of food.” But full rations are rarely met. Food consumption tends to tail off in spring, as stocks from the previous year dwindle. The lean months last until autumn, after the harvest. Meat is distributed only during holidays, such as last week’s anniversary of the birth of Kim Il Sung, the nation’s founder. To supplement diets, CFK distributes tons of donated canned meat to TB rest homes, accounting for 20% to 70% of patients’ protein consumption. It also sends greenhouse kits, seeds, and soil inoculants.

Another driver of the TB epidemic is an inconsistent drug supply, especially before WHO opened its country office in Pyongyang in 2001. North Korean firms produce at least two drugs used to treat TB—isoniazid and streptomycin—but quality is uncertain and administration is uncontrolled. Stanford researchers have bought both in hotel convenience stores. The health ministry has no funds to import TB medications, so its supply of reliable drugs now comes primarily from the Stop TB Partnership’s Global Drug Facility, which operates out of WHO, and the Eugene Bell Foundation, a humanitarian organization based in Washington, D.C.



A deep bond. Kim Ha’s gift of pheasant eggs touched Heidi Linton.



Poor compliance with therapy has spurred TB rates higher. TB drugs must be taken daily for up to 2 years; abandoning a regimen in midstream breeds resistance and treatment failure. To ensure compliance, health departments around the world have implemented directly observed therapy, short-course, or DOTS, in which health workers watch patients take their medicine. North Korea began practicing DOTS routinely only about 10 years ago.

Rampant resistance

A watershed moment for Linton came in the spring of 2007, on a visit to the Hwangju TB Rest Home. The director, Kim Ha, presented her with several bluish-green pheasant eggs. “His hands were hot with fever,” Linton recalls. “He was trying not to cough on us.” Ill with TB, Kim had spent 4 days in the hills gathering the eggs—the most precious gift he could muster—to present to the CFK team. “He needed that protein. His staff, his patients needed that protein, way more than we did. I was in tears,” she says. “Some people back in the States ask me why we do this, helping ‘those ungrateful people,’” Linton says, voice breaking. She tells them about Kim’s gesture. “If that’s not grat-

itude, I don't know what is."

Six years later, Kim, 58, is enduring his fifth bout of TB. This time it's MDR. "I was going to retire," he tells Linton, "but the food you sent us gave me the strength to work." After a stint in the army, Kim entered medical school and worked at Hwangju County TB Hospital before being assigned to the rest home. He vows to finish building an MDR ward here at the sanatorium before hanging up his stethoscope. MDR is a burgeoning problem in Hwangju, Kim says: "It's a big worry." He attributes the increase to frail health and poor nutrition, failure to adhere to drug regimens, and heavy alcohol consumption.

Linton has a donation just for Kim: three pairs of handmade wool mittens. "The ladies didn't know how big your hands are, so they made these in different sizes," she says. "A lot of people are praying for you." Kim says thanks, and with a wan smile asks whether CFK can also donate a motorcycle.

Each day before breakfast, Linton's CFK team gathers in their hotel for devotions. On Sunday mornings, they attend services at one of the three Christian churches in Pyongyang. CFK was founded after evangelist Billy Graham's visits to North Korea in 1992 and 1994 paved the way for Christian groups to come in. "It took a while for us to understand each other," says Schoolnik, who is not Christian. But Linton, he says, has been instrumental. "She has enormous on-the-ground experience and is extremely well-respected," he says. "I'm absolutely sure we could not have worked in North Korea without her."

It will take more than prayer to tackle North Korea's MDR problem. DPRK's health ministry estimates that up to 15% of TB patients



Mounting toll. A health official logs sputum samples of suspected MDR patients in Unpa.

Paek Myong Hwa, director of the Suan TB rest home, says that in his region the number of suspected MDR cases is "stable," but that patient health "is getting worse." Scientists can only guess which strains are circulating. "We have no idea what the drug-resistance patterns are," England says. One of her main tasks these days is to help NTRL researchers implement drug-susceptibility tests, or DSTs. "As soon as they can independently do their DSTs, it will be a big step," she says.

Even for top-notch public health systems, MDR is a heavy burden; therapy lasts 2 years and costs as much as 200 times that of the four-drug combo used to vanquish susceptible strains. Modern care cannot prevent

one in five MDR or XDR patients from succumbing to the disease. Since 2008, the Eugene Bell Foundation has taken the lead on MDR in North Korea; it sends sputum samples from suspected MDR patients to Seoul for DST panels, and it tailors drug regimens to individuals. MDR patients are treated at district-level rest homes. But it can take days or weeks before they get appropriate MDR drugs, and there aren't nearly enough of these to go around. During that gap, some rest homes give suspected MDR patients traditional medicine, such as a tincture of *wonchuri*, or day lily.

As North Korea's TB epidemic gained momentum, the need for a modern lab for diagnoses and uncovering drug-resistance grew more urgent. By 2008, North Korea was the only country with a high TB burden lacking a modern facility for culturing the bug and probing drug resistance. In January that year, the Stanford-led Bay Area TB Consortium hosted a delegation from DPRK's health ministry; later, the Nuclear Threat Initiative awarded the consortium a grant to purchase state-of-the-art equipment to establish an NTRL at District #3 TB Prevention Hospital in Pyongyang (*Science*, 12 March 2010, p. 1312). Others that have pitched in to keep it going include the Richard Lounsbury Foundation, the Walter H. Shorenstein Asia-Pacific Research Center, and Zero TB World.

Work on the lab never broke stride, even during acute political tensions that followed DPRK missile and nuclear tests, military drills, the sinking of South Korea's *Cheonan* naval vessel in March 2010, and the North's shelling of Yeonpyeong Island that November, which killed four South Koreans. "Our North Korean colleagues have overcome tremendous odds," Linton says.

One step forward

In a room with negative air pressure to prevent airborne microbes from escaping, a reed-thin technician, Kim Myong Hyok, reaches into a centrifuge and removes plastic Falcon tubes from a bucket one by one. In each tube is a sample of sputum that had been treated with n-acetyl cysteine to break up the mucus and sodium hydroxide to kill off microbial flora other than mycobacteria. Malachite green, added to inhibit fungal growth, gives the samples an alien look.

After moving the centrifuge tubes to a biosafety cabinet, Kim takes one and decants off the supernatant, then dilutes the pellet in phosphate buffer. ("pH is important. Many little nuances are important," England says.) Kim pipettes some of the suspension onto the surface of a greenish gel in a test tube. Mycobacteria thrive on the Lowenstein-Jensen medium, a protein-rich goo made from antibiotic-free eggs. Another portion of the sample will later be smeared on a slide. "In the begin-



Lending a hand. CFK has distributed greenhouses and water systems to TB rest homes.

fail first-line treatment, which suggests that they are actively infected with drug-resistant strains. Based on those figures and drug resistance patterns elsewhere in Asia, North Korea could have as many as 15,000 people with drug-resistant TB, England says. Many, she says, are undiagnosed, untreated, or being treated inappropriately. Few MDR patients are isolated from others in TB wards or from healthy people. In the TB rest homes, "it's worrisome that a patient being treated for drug-susceptible TB could become infected with a drug-resistant strain by being exposed to another patient," says Edward Desmond, head of the mycobacteriology and mycology laboratory section at the California Department of Public Health in Richmond. "In the absence of data on the prevalence of drug resistance, it's impossible to calculate how serious this problem is," says Desmond, who has visited North Korea and worked on the TB project on his own time.

ning here we used old-school, acid-fast staining," England says. They've since graduated to a fluorescent stain that lights up the bacterial rods bright yellow.

The fact that this scene could be happening in any modern TB lab anywhere in the world is encouraging to England. She cut her teeth in the region in Zhengzhou, China, where from 2010 to 2011 she trained TB researchers involved in a U.S.-sponsored clinical trial. She next spent a year in Ulaanbaatar, where she designed labs for Doctors Without Borders and assisted Mongolia's National TB Reference Laboratory. The stint revealed to her the extent to which the disease blights impoverished communities in developing countries to this day. "Mongolia opened my eyes," she says.

England's role in Pyongyang is broader still. Besides training NTRL's 14 staff members, she is assisting the lab in strengthening diagnostics, establishing quality assurance for lab activities, planning a drug resistance survey, developing regional labs with WHO, and preparing for accreditation. "The lab is a big help for our people," says Kim Hyon Chol, an NTRL microbiologist who is working with England on the resistance screening. "We're developing a cadre of talented, hard-working young people," Schoolnik says. "There is a true sense of trust on both sides."

Last month, NTRL received a GeneXpert machine for rapid diagnosis of rifampicin resistance. MDR strains are defined as those resistant to isoniazid and rifampicin, the two most powerful drugs of the 6-month treatment for drug-sensitive TB. (The other two drugs are ethambutol and pyrazinamide.) Rifampicin resistance is used to diagnose MDR, because it almost invariably takes longer to develop than isoniazid resistance.

"We're moving into molecular testing," England says. But bringing NTRL up to speed has been slow going, she says. "Progress continues at a snail's pace, which is okay as long as we keep moving forward."

Before Stanford came on the scene, the only exposure to Western research practice that the TB specialists here had received was in southern India, from 2002 to 2004. With support from WHO, 19 Koreans each spent 15 days training at the National Institute for Research in Tuberculosis in Chennai. "It was difficult to judge how much they had understood and digested. They were not very communicative," says the institute's director, Soumya Swaminathan. A physician and a mycobacteriologist from Chennai paid visits to North Korea between 2004 and 2006. The doctor reported that patients "seemed severely malnourished," Swaminathan says, and the scientist "was frustrated" over the lack of a regular power supply that thwarted setting up TB cultures.

Power woes continue to bedevil research facilities in Pyongyang, including NTRL. Last November, when power to the lab was cut, Schoolnik asked the Koreans why they hadn't switched on their backup generator. They



Identifying gaps. "We have no idea what the drug-resistance patterns are," says Kathleen England, beside an NTRL sample incubator.

and didn't want them to move in," Ko says. The idea was abandoned.

That paid off for Unpa. In November 2010, CFK installed a water tank up the hill from the sanatorium. Unpa's residents, Ko says, were glad that the rest home had stayed put: The tank supplies fresh water to the nearby village. Next on Unpa's wish list is a wind turbine to generate electricity; power is so spotty that the rest home isn't even fitted with light bulbs.

Back in Pyongyang, the CFK team drives past a diplomatic compound in Sadong District, in the northeast, and turns down a pothole-pocked lane packed with dilapidated dwellings. It's a poorer corner of the capital that few foreigners glimpse. Our van pulls up at a pink two-story building: the pediatric ward of the Pyongyang District #3 TB Prevention Hospital. It has a capacity of 100 patients, but today there are only 14 children, ages 6 to 15, and one infant. "As it gets warmer, we expect to have more," says the new director, Ri Sang Il. TB patients come out of the woodwork in spring, after being cooped up all winter huddling to stay warm, Linton says.

In some ways, Ri says, children are easier to treat than adults. "They are eager to follow what the doctors recommend," he says. "But they can also be naughty," one of Ri's colleagues chirps up, before adding, somberly, that the kids spend about 4 months on average in the ward and get really homesick. About a third of the children have digestive problems from the harsh TB drugs.

On this early spring morning, the pediatric ward is chilly. Nurses lead the children out into the sun for fresh air and warmth. The kids are wearing white pajamas with blue pinstripes over their clothes. "If people back home could spend 10 minutes here, driving through some of the areas we drive through, walking through patient wards, I think they'd get it," Linton says.

She sees reason to hope. The scientists and administrators, she says, "long for something that's real. They long for truth." But Linton knows that it will take more than a modern lab to bring TB to heel. "We need to be infused with a passion to do whatever it takes to stop this disease, and reverse it," she says, "for the sake of their people and this region and the world."

—RICHARD STONE

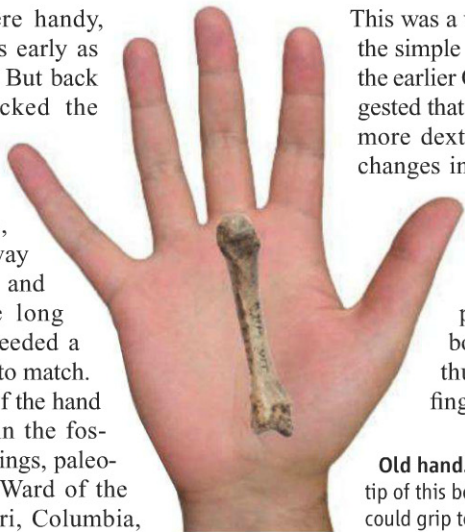


Out into the light. Young TB patients get some fresh air at a pediatric ward in a northeast district of Pyongyang.

When Early Hominins Got a Grip

Human ancestors were handy, crafting stone tools as early as 2.7 million years ago. But back then their hands lacked the crucial adaptations for tool use seen in living people. About 1.7 million years ago, hominins found a way to make a better tool, and anthropologists have long suspected that they needed a different kind of hand to match. But the fragile bones of the hand are rarely preserved in the fossil record. At the meetings, paleo-anthropologist Carol Ward of the University of Missouri, Columbia, announced a modern feature in a rare 1.4-million-year-old hand bone from Kenya, filling a 1-million-year gap in the fossil record and showing when key adaptations to toolmaking arose. “The changes in the hand seem to be occurring much earlier in time [than fossils showed before] and are associated with the evolution of *Homo erectus*,” Ward said in her talk.

About 1.7 million years ago, *H. erectus* began to make tear-shaped hand axes in the so-called Acheulean style, in which the blade is flaked symmetrically on both sides.



This was a vast improvement over the simple choppers and flakes of the earlier Oldowan style and suggested that hominins were getting more dexterous with tools. But changes in the hand bones have been more obscure. No one has known just when our ancestors developed a complex of changes in the bones at the bases of the thumb, index, and third finger that allow a force-

Old hand. The point at the bottom tip of this bone suggests its owner could grip tools precisely.

ful precision grip. That grip, in which the fingertips and thumb press against an object in the grasp, such as a hand axe, is anatomically demanding: The hand has to curve to grip the tool, but it also has to stiffen at the base of the thumb and in the midhand, to stabilize the hand and dissipate force.

Modern humans and Neandertals have this complex of traits, as does the 800,000-year-old *H. antecessor* from Atapuerca, Spain. But members of *Australopithecus*, which lived about 1.9 million to

4 million years ago, do not. Thus, this refined precision grip arose sometime between 1.9 million years ago and 800,000 years ago. But when? No one knew, because no fragile hand bones had been discovered during this time. “There’s a huge gap of 1 million years in the fossil record for hands,” Ward says.

Then, in 2010, paleoanthropologist Frederick Kyalo Manthi of the National Museums of Kenya in Nairobi found a bone at the newly discovered site of Kaitio on the edge of Lake Turkana in Ethiopia. The bone, the third metacarpal, sits in the middle in the hand, extending from the knuckle of the third finger to the wrist. Although it is just a single bone, it has a key feature—a bony point or styloid process—at the bottom. This point is “associated with making and using tools,” Ward says, because it buttresses other bones to stabilize them and so allows a forceful precision grip.

Ward’s team can’t say for sure which *Homo* species the bone represents, but they suspect that it was large-bodied *H. erectus*, our direct ancestor, who lived near Lake Turkana during this time and is thought to have left the Acheulean tools. The bone was larger than the same bone in the hand of co-author J. Michael Plavcan of the University of Arkansas, Fayetteville, “who has really big hands,” Ward notes, implying a big individual.

The fossil shows that by the time the Acheulean was well established, the precision grip was, too. “The discovery is significant because it corroborates evidence from

PALEOANTHROPOLOGY SOCIETY | 2–3 APRIL | HONOLULU

Following the Males’ Trail, 1.5 Million Years Later

HONOLULU—Fossils can say a lot about what human ancestors looked like, but their evidence is indirect when it comes to how hominins behaved. That’s why rare finds of hominin footprints generate such excitement: They capture ancient human activity at a moment in time. But only a handful of such tracks are known.

At the Paleoanthropology Society meeting here this month, a team described 1.5-million-year-old footprints of at least six individuals walking together across the sand at Ileret, Kenya. Coupled with experiments with living people, the researchers concluded that the size and direction of the prints suggest a party of males out

hunting or on patrol, an example of male coalitional behavior also seen in chimpanzees, says team leader Brian Richmond of George Washington University (GWU) in Washington, D.C.

The team had earlier reported other footprints at Ileret, (*Science*, 27 February 2009, p. 1197), which showed that by 1.5 million years ago hominins had evolved a nearly modern foot. Richmond reported here on two new footprint trails: one headed east and the other west. The prints, made in mud near a river, were well preserved by later mud flows that quickly covered them and then hardened. The eastbound prints were made by six to eight individuals; the west-

bound prints were made by at least five. Some of the westbound prints were partly superimposed on the first set and could have been the same individuals coming back, Richmond said.

Unlike TV detectives, who can tell exactly which shoe a culprit was wearing from their prints, the traces of barefoot hominins have to be interpreted cautiously, Richmond said. A footprint in sand is not a mirror image of the foot that made it, because it also reflects the type of sediment and how fast the maker was traveling.

So in a companion talk, GWU Ph.D. student Kevin Hatala detailed experiments with the local Daasanach people, who tradition-

CREDIT: MATT TOCHER AND CAROL WARD

other parts of the hand that the hominin hand may have become adapted to stone toolmaking with the appearance of the Acheulean,” says paleoanthropologist Mary Marzke of Arizona State University, Tempe, who was not a co-author. —A. G.

Ardi's a Hominin—But How Did She Move?

Ardi, the oldest and most complete skeleton of a potential human ancestor, has been a leading lady of human evolution ever since she got top billing on the cover of *Science* in 2009. But while everyone praised the completeness of the partial skeleton, some researchers reserved judgment on whether *Ardipithecus ramidus* was actually a hominin, or upright member of the human family.

At the meetings, a series of talks and posters gave Ardi new recognition as a hominin. Researchers who have had their first look at fossils or casts of the 4.4-million-year-old fossils agreed with the discoverers that Ardi walked upright—albeit in a weird way—and that features in her teeth and skull make her a primitive member of the human family. But debate raged about whether she also climbed vertically in trees like an ape.

In 2009 (*Science*, 2 October 2009, p. 36), a team of 47 researchers co-led by Tim White of the University of California, Berkeley, showed that chimp-sized Ardi walked upright, planting her feet flat on the ground. Although she still clung to the safety of trees, the team suggested that she didn't climb ver-



Making headway. The bones at the base of Ardi's skull suggest that she had human traits.

apes to accommodate a more forward position of the spinal cord.

Others found Kimbel's talk convincing. “When I saw the cranial base, it struck me that it was reasonable as a hominin,” says paleoanthropologist Eric Delson of the City University of New York. “Above the neck, I'm convinced.”

tically or swing through the branches like a chimp, but moved slowly on all fours when on top of branches, with an opposable big toe to grasp limbs. But other researchers continued to debate whether *Ar. ramidus* was part of the human lineage (*Science*, 29 January 2010, p. 532).

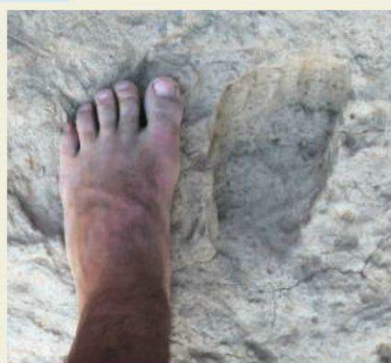
In one talk, William Kimbel of the Institute of Human Origins at Arizona State University, Tempe, working with White's team, used the cranial base—the bones that form the base of the inside of the skull, around the opening for the spinal cord—to support Ardi's status as a hominin. Kimbel, an expert on Lucy's species, *Australopithecus afarensis*, noted resemblances between that species and Ardi, which is half a million years older but from the same region in Ethiopia. For example, both have a shorter and wider cranial base than an ape's. “What struck me was just how spread out the landmarks were around the center of the cranial base,” Kimbel says. “In apes, they are crowded together in the middle.” Hominins have a wider base than

Below the neck, interpreting Ardi's anatomy is more complicated. The position of one key hand bone, the capitate in the center of the wrist, supports the idea that Ardi's wrist was flexible, allowing her to walk on flat hands and feet atop branches, reported anatomist Michael Selby of the Philadelphia College of Osteopathic Medicine, Georgia Campus, in Suwanee. But that view was challenged by paleoanthropologist Caley Orr of Midwestern University, Downers Grove, in Illinois. Using a 3D analysis of the wrist bones, he found that Ardi's wrist was too rigid to put her weight flat on her hands, suggesting that she moved through the trees in another manner, perhaps by vertical climbing as chimpanzees do.

So while Ardi is on more secure footing as an upright walking hominin, the focus has shifted to what she did off the ground. “The big debate is how it was moving in the trees,” says paleoanthropologist Will Harcourt-Smith of the American Museum of Natural History in New York City. —ANN GIBBONS



Footsteps on the sands of time. Comparing ancient and modern footprints at Ileret, Kenya (above), gives insights into early human behavior.



ally walk shoeless. The researchers asked 20 Daasanach men and 20 women to walk and run barefoot across a strip of sand, moistened to various degrees, next to the Ileret site. The length of their footprints correlated well with the length of their feet, but print width was a poor predictor of actual foot width. The footprint dimensions were good predictors

of height and reasonably good predictors of body weight, Hatala found.

Three hominins lived near Ileret around that time: *Homo erectus*, *H. habilis*, and the australopithecine *Paranthropus boisei*. The prints were all too large to have been made by the smaller *H. habilis* or by females, the team concluded, and must have been made

by males of either *H. erectus* or *P. boisei*.

Such gatherings of males are seen “in humans that are hunting or tracking, or groups of male chimps out on patrol,” Richmond says. “This male coalitionary behavior was probably represented in the common ancestor of humans and chimps.”

Others at the meeting expressed cautious enthusiasm. “Footprints are cool,” says Ariane Burke, an anthropologist at the University of Montreal in Canada. But “interpreting social behavior based on footprints begs for extreme caution,” says Zeray Alemseged, an anthropologist at the California Academy of Sciences in San Francisco. Anthropologist Susan Larson of the Stony Brook University School of Medicine in New York agrees, but adds that “at least this speculation has some basis in fact.”

—MICHAEL BALTER

LETTERS

edited by Jennifer Sills

The Brazilian Adirondacks?

THE NAME OF THE SERRA DA MANTIQUEIRA MOUNTAINS, TRANSLATED FROM THE INDIGENOUS Tupí-Guaraní language as “Weeping Mountains,” underscores their historical importance as a source of water for southeastern Brazil. Mantiqueira harbors outstanding sociocultural history, and its high peaks form a key wildlife corridor within the Atlantic Forest Biodiversity Hotspot (1). The striking scenery and proximity to Rio de Janeiro and São Paulo (17.6 million inhabitants combined) offer an opportunity to consider new models in conservation and sustainability.

In 2006, the Brazilian Ministry of the Environment created the Mantiqueira Mosaic, a network of public and private conservation units, to enhance biological conservation and local welfare. However, the implementation of most conservation units has been delayed and many still lack a management plan. For instance, the creation of the largest park (Parque Nacional Altos da Mantiqueira), which will cover 86,000 hectares of high peaks (2), has been stalled since 2010. Brazil's booming economy, and the 40% predicted increase in agricultural land use in the next decade (3), will pose serious threats to the remaining ~10% of the Brazilian Atlantic Forest (4).

In the 19th century, the Adirondack Mountains, adjacent to New York City and Albany (8.4 million inhabitants), faced a strikingly similar scenario due to logging and mining activities,

leading to creation of the Adirondack Park in 1892 (5). Along with the Algonquin Provincial Park in Canada, the Adirondacks serve as a key wildlife corridor in North America (6). The Adirondack Park successfully manages an integrated mosaic of private and publicly owned lands, supports sustainable land use, regulates recreational activities, and promotes ecotourism and education (5).

The conservation challenges for the Mantiqueira range and Adirondacks are similar, despite being proposed more than a century apart. The future of Brazil's most prominent mountain chain as wilderness now depends on accelerated implementation of the conservation network by the Brazilian Federal Government so that the Mantiqueira Mosaic can promote biodiversity conservation and environmental stewardship through sustainable land use and citizen access.

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Japan's Lagging Gender Equality

JAPAN, ALTHOUGH WELL QUALIFIED AS A country that promotes scientific advancement, lags behind other developed nations in gender equality. The percentage of female professionals trained in science, technology, engineering, and mathematics (STEM) is at 13.8%, the lowest among developed countries (1). Why is Japan so slow in maximizing the potential of female scientists in STEM? Survey results (2, 3) indicate that there are too few women in positions of authority who can help younger women with career enhancement. Furthermore, male scientists show an unconscious bias when evaluating their female colleagues. Finally, female scientists often avoid competition and underestimate their ability, leading to passivity when seeking leadership roles.

In 2009, the Ministry of Education, Culture, Sports, Science and Technology in Japan initiated the program “Supporting Positive Activities for Female Researchers.”

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

CREDIT: ORLANDO MOHALLEM



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IBI prize essay

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The 5-year program's goal is to accelerate the numbers of female scientists and their promotion rate at 12 research universities. A midterm evaluation of the program's impact showed a substantial increase in female faculty, particularly in positions of greater

responsibility in several universities (4, 5). The program gives university leaders a clear path to unlocking the potential of female scientists and helps pave the way with affirmative action, reserving positions for women.

These government measures will only

have a long-term effect on the ratio and roles of women in STEM fields if the academic climate and leadership changes in Japan. This challenge will require proper enforcement of regulations by deans and department leaders.

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Readers' Poll Results

Funding Environment

In his 15 March Editorial "Am I wrong?" (p. 1252), B. Alberts lamented the decline of research funding available in the United States and predicted that misguided short-term decisions could severely affect the nation's future. He asked scientists to write in and tell him whether his impression was wrong. In a related poll, Alberts asked:

To what extent is the current grant funding environment undermining the intellectual environment and creativity in your institution?

More than 100 readers commented online (see all comments at <http://comments.sciencemag.org/content/10.1126/science.1237434>), and more than 1300 voted in the poll, from countries all around the world. Here are your results.

A selection of your thoughts:

You are not wrong, but no one should be allowed to put demands on the treasury without some consideration of the budget and national debt.

—Chuck Coltharp

I share your concern about the national debt, [but]...since the end of World War II, somewhere between 50 to 85% of the economic growth in the country has come from scientific and technological advances....The very nature of scientific research makes its returns uncertain, so companies can't afford to push the frontiers themselves, only we the taxpayers can do that. ...

—Jake Searcy

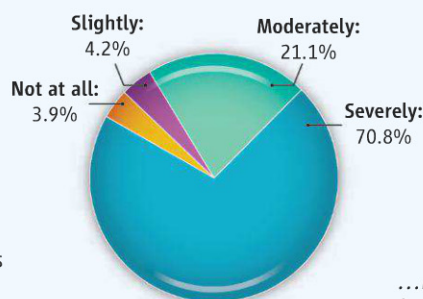
The NIH budget needs to stop being viewed as a welfare expenditure. This means making the NIH budget independent of [the Department of Health, and Human Services] and recognizing the NIH as part of the infrastructure of the biomedical industry. It should be funded like highways or airports that are supported by fees levied on the commerce they support.

—Mark Ginsberg

The lack of funding for fundamental research in the U.S. (and in Canada, where I live) appears to be directly linked to the politicians' and the general public's concentration on short-term goals and benefits (called "innovations" in Canada), and their lack of understanding of the benefits that ultimately accrue from basic research....

—Don Baker

The biggest problem for academic scientists is the divestment of state support, which has forced them to rely on grants for their salaries. This



drives them into the grant writing frenzy that we see now. It also diminishes their productivity and proliferates mediocre and dubious science, a waste of federal funds. Dissociating research dollars from salary support ensures less stress, greater productivity, and higher quality. Considering that salaries form a big chunk of any grant, one could also run a lab effectively with smaller grants....

—Sukumar Vijayaraghavan

...Periodic downturns in funding provide an opportunity to weed out the less than effective.... In tough times, one redoubles the effort to exploit the stock of recently acquired new knowledge, rather than generate more new knowledge and leave it unexploited. This makes sound economic sense....

—Michael Kelly

It is a pity that ... the financing of science is still based on the national partitioning of the world ... There is a World Bank; why can't we have a world science fund replacing national funding systems?

—Hans Rogl

That is true! ...International colleagues are increasingly [saying] that they plan to return to their own countries because their countries can provide a better research environment, such as financial support, laboratory apparatus, and living conditions. [This] used to be the advantage of the U.S....

—Xu Hou

You are certainly not wrong, but sadly, U.S. scientists are still better off than here in New Zealand....

—Jon Hickford

If this is the grim situation in the U.S., you don't want to think about Spain: ...We have trained in the past two decades the best cohort of young people we ever had. Now...the governmental cuts in science spoil all the recent efforts....

—Francisco X. Real

...In my country [Italy] ... most of the research is done thanks to people who don't get any money for their efforts....

—Christian Marinaccio

Bruce's Editorial is right on the dot. Germany is doing very well in job creation, thanks to the massive investment in education, research, and technology....

—Amar Nath

Polling results reflect only the votes of those who choose to participate; they do not represent a random sample of the population.

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Strategy Behind Science Policy Decisions

PRESIDENT OBAMA'S ANNOUNCEMENT OF the BRAIN Initiative has caused quite a stir. Many scientists welcome this large new thrust in neuroscience, whereas others question the technological feasibility of the project.

Whether or not the project becomes a major scientific success, it is an instructive example of how science policy decisions are

made. A relatively small group of scientists and foundation people were able to create a compelling vision and then persuade senior policy-makers to embrace it.

In the broadest sense, it is good to see any new investment in basic science. However, is the BRAIN Initiative the very best use of the approximately \$100M/year research investment? In terms of overall global good, would the money be better spent on malaria research? On better antibiotics for drug-resistant bacteria? On early detection of tumors?

The BRAIN Initiative highlights the fact that we do not have a systematic approach to assessing the nation's need for research and development.

RUDY JULIANO

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TECHNICAL COMMENT ABSTRACTS

Comment on "In Monkeys Making Value-Based Decisions, LIP Neurons Encode Cue Salience and Not Action Value"

William T. Newsome, Paul W. Glimcher, Jacqueline Gottlieb, Daeyeol Lee, Michael L. Platt

Leathers and Olson (Reports, 5 October 2012, p. 132) draw the strong conclusion that neurons in the monkey lateral intraparietal (LIP) cortical area encode only cue salience, and not action value, during value-based decision-making. Although their findings regarding cue salience are interesting, their broader conclusions are problematic because (i) their primary conclusion is based on responses observed during a brief interval at the beginning of behavioral trials but is extended to all subsequent temporal epochs and (ii) the authors failed to replicate basic hallmarks of LIP physiology observed in those subsequent temporal epochs by many laboratories. Full text at <http://dx.doi.org/10.1126/science.1233214>

Response to Comment on "In Monkeys Making Value-Based Decisions, LIP Neurons Encode Cue Salience and Not Action Value"

Marvin L. Leathers and Carl R. Olson

Newsome *et al.* question neither our key result, that large-penalty cues elicited stronger responses than small-penalty cues, nor our key conclusion, that neurons early in the trial signaled cue salience and not action value. Instead, they focus on subsequent neuronal activity. The patterns of delay-period activity that they note can be explained by reference to experimental methodology.

Full text at <http://dx.doi.org/10.1126/science.1233367>



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To Improve Public Policy

Brian Baird

The impact of scientific discoveries has never been greater, but the ability of science to affect public policy, at least in the United States, is foundering. Which raises the question, how can science and scientists better inform public policy? Nancy Cartwright and Jeremy Hardie's *Evidence-Based Policy: A Practical Guide to Doing It Better* and Charles Manski's *Public Policy in an Uncertain World: Analysis and Decisions* attempt to answer this question. Consistent with their internal logic, they succeed and fall short to different degrees and for different reasons. Setting aside, for the moment, the much deeper, more troubling, and in these works largely unasked question of whether policy-makers and citizens actually want science to inform policy, it is worth examining each book from the perspectives of both academia and applied policy.

For policy-makers and students of policy, the more useful of the two books is likely to be that by philosophers Cartwright and Hardie (London School of Economics). The authors' particular concern is the elevation of and dependence on randomized controlled trials (RCTs) as the gold standard against which the quality of all research must be weighed and by which social policy and practice of everything from education to health care increasingly must be guided. The problem, as presented, is not with the internal validity of RCTs but with the way in which findings of a given RCT are applied to different situations by policy-makers with inadequate attention to all the factors that can profoundly influence the outcome in the new setting. The authors identify this challenge as how one gets from the evidence that something worked "there" (i.e., in the circumstances of a specific RCT or even multiple RCTs) to the conclusion that it will work "here" (i.e., in the

new circumstances in which a policy-maker seeks to solve a problem that, at least on the surface, appears to be similar).

Evidence-Based Policy
A Practical Guide to
Doing It Better

by **Nancy Cartwright and
Jeremy Hardie**
Oxford University Press,
Oxford, 2012. 208 pp. \$74.95,
£45. ISBN 9780199841608.
Paper, \$18.95, £11.99.
ISBN 9780199841622.

**Public Policy in an
Uncertain World**
Analysis and Decisions

by **Charles F. Manski**
Harvard University Press,
Cambridge, MA, 2013.
215 pp. \$39.95, £29.95, €36.
ISBN 9780674066892.

rather here, lies the greatest value of the work.

The differences between internal and external validity and efficacy versus effectiveness are often discussed by scientists but too rarely appreciated by policy-makers and, according to the authors, understood too simplistically and largely impractically by researchers and policy-makers alike. After a lengthy, and (for a policy-maker) unneces-

sarily technical, discussion of the many factors that can differ between experimental and applied worlds, the authors offer a number of more practical suggestions and examples of how this translational decision process can be made.

Even though fairly short, *Evidence-Based Policy* is redundant in parts, and the key points could be stated more simply and succinctly. Nevertheless, it would be worthwhile for policy-makers and well-intentioned researchers to consider carefully the caveats Cartwright and Hardie raise before recommending that a "plug and play" intervention "proven" to be effective in one setting be applied formulaically elsewhere. Failure to do so, as the authors point out, is likely to squander public resources without achieving the desired outcomes. Worse still, unintended and undesired consequences may result from well-intended and what appeared, superficially, to be evidence-based interventions.

Public Policy in an Uncertain World focuses on a different but related science-policy shortcoming. Manski (an economist at Northwestern University) argues that scientists too often offer advice that conveys what he calls "incredible certitude"—that gives an impression the scientific evidence is both more conclusive and more precise than is actually warranted. At the same time, Manski observes that scientific expressions of uncertainty and recognition of the counterfactual are too rare. He cautions, "When researchers overreach, they not only give away their own credibility, but they diminish public trust in science more generally." As an antidote to incredible certitude, Manski advocates, among other recommendations,

greater reporting of "credible interval scoring" versus single point estimates.

Like Cartwright and Hardie, Manski directs much of his critique to the overreliance on RCTs and the challenge of extrapolation from research findings to policy applications. To this, he adds commentary on the shortcomings of peer review and the inherent logical and practical limitations of various methodological and research design features ranging from subject selection to randomization to choice of statistical analysis and reporting.



The reviewer, a member of the National Academy of Sciences Division of Behavioral and Social Sciences and Education Advisory Committee, was the U.S. Representative for Washington's 3rd District from 1999 to 2011. E-mail: brianbairdphd@gmail.com

To illustrate his points, Manski cites examples of the gaps between economic theory, research, and policy as well as social interventions, including evaluation of the effects of different criminal sentencing approaches. Much of his discussion centers on long-standing debates within economics and decision theory.

To academic readers steeped in these disciplines, his account is likely to be of some interest. It includes many useful and important insights (for example, the distinctions among policies based on the principles of “maximin,” “minimax,” and “adaptive minimax” regret) that have substantial implications for real-world policy. Unfortunately, from a practical perspective, those tend to get lost in more technical and polemic discussions of the writing.

Such criticism may seem like nitpicking, or even an irrelevance, to the academic community. However, if part of the stated goal of the work is to inform policy decisions, it is important that the informational process be applicable and accessible to the target audience.

Herein lies one of the shortcomings of both works and, indeed, of most related recent books on the subject. Just as it can be challenging to translate research findings from the there of one study to the here of a new application, the translation of theory and findings from the there of academia to the here of policy is more difficult than most academics or policy-makers appreciate. Speaking as a former member of Congress and a former academic researcher, I know this too well from both sides. Coming from that dual perspective, I found both books to be interesting, if sometimes unnecessarily challenging. Unfortunately, it seems likely that the odds are against their recommendations having the broader applied effects the authors seek.

Such a lack of impact would be a particular shame in two key areas: Manski provides thoughtful and very practical suggestions for how and why the U.S. Food and Drug Administration should alter its approach by implementing a process of “adaptive partial drug approval.” Cartwright and Hardie put forward analysis and models that should (in simpler form) be integrated into, and prominently featured as guidance in, all research warehouses—to avoid purveying evidence-based policy recommendations without sufficient attention as to how they should, or should not, be applied.

Finally, any effort to improve how science informs policy must deal with the disturbing reality that a great many policy-makers in the United States today don’t necessarily

want science to inform policy to begin with. This, I am deeply sorry to say, is true even on the House of Representatives Science and Technology Committee. *Evidence-Based Policy* and *Public Policy in an Uncertain World* could contribute to the committee’s understanding and deliberations. However, reading both books, I could, sadly, just as easily imagine key points being extracted or misapplied by certain committee members to justify the very unscientific processes and conclusions that now distort so much of how Congress functions. That is certainly not the fault of these well-intentioned authors, but it may well limit the applicability of their lessons.

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EXHIBITIONS: MATERIALS SCIENCE

Transit Shadows

Thomas Junier¹ and Pilar Junier²

The deafening sound of Etienne Krähenbühl’s *Bing Bang* greets visitors to *SUPRA100*, an exhibition of his work currently at the Museum of Natural History of Neuchâtel. Collaborating with the Swiss National Research Program in Materials with Novel Electronic Properties at the University of Geneva, the artist has found a way to present to the public the prospects of superconductivity, a research domain involved in fields as diverse as medicine, communication, and transport technologies. His sculptures demonstrate fresh ways of exploring the mechanical and artistic possibilities of metal alloys—in particular, a superelastic nickel-titanium alloy that he used in many of the pieces on display.

Bing Bang may be the star of the exhibition, in a more than metaphorical sense. It is an array of 850 iron rods of different lengths, each hanging from a wire. At rest, the structure forms a placid sphere. When squeezed together and suddenly released, however, the work seems to spring to life: its complex oscillations irresistibly evoke breathing or a heartbeat; despite being made of metal, its wobbling shape is uncannily organic. But the work’s

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Etienne Krähenbühl’s *Bing Bang* (2010).

scope is far greater than the living world: it might as well stand for variable stars or simply the expanding (and contracting) universe. And it chimes. That a seemingly chaotic jumble of iron bars can produce a pleasant sound is a real wonder. Much has been speculated about the music of spheres; through *Bing Bang*’s cosmic music, one can almost hear the fossil radiation. The secret lies in technology: the wires are superelastic, and with ordinary wires the magic disappears.

Pieces such as *Viagra Cube*, which makes an evocative link between temperature and the rigidity of the material, illustrate interplay among temperature, material memory, and artistic expression. Through his association with material scientists, Krähenbühl has developed unique pieces that blur the frontier between expression and creation. One of the most impressive ones, *SUPRAsphère*, consists of a sphere mysteriously floating over a surface that hides the supraconductive engine moving randomly underneath.

After visiting *SUPRA100*, you might wonder if the universe started with the big bang or if it was instead born from the inventive mind of a physicist considering the possibilities of elements and materials. The exhibition reminds us that scientists are hidden artists and that artists can be hidden scientists, too.

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SUPRA100

Le temps suspendu

Muséum d'Histoire Naturelle
Neuchâtel, Neuchâtel,
Switzerland. Through 20
May 2013. www.museum-neuchatel.ch/new/navigation.php?cat=5

SCIENCE AND LAW

Human Subjects Protection and Research on Terrorism and Conflict

Brian A. Jackson,^{1*} Tora K. Bikson,² Patrick P. Gunn³

The last decade has seen considerable expansion in research on terrorism and conflict. People have studied root causes, group behavior, and different approaches to counter such acts (1). Some of the best cases involved direct interaction with current or former terrorists, producing important results that, for example, replaced caricatures of terrorists as pathological killers with nuanced models of what drives individuals to join such groups and even sacrifice themselves intentionally for a cause.

We discuss how current legal developments raise complications and may limit the ability of researchers to work on these topics. These issues are particularly important for U.S. research policy, as the Department of Health and Human Services (HHS) is considering major changes in the way human subjects are protected (2).

Efforts to understand terrorism have included internal government programs—from basic research to mission-focused intelligence analysis—and extramural projects involving academic institutions, research organizations, and firms hired to collect data to inform mission decisions. Depending on how these efforts are defined and who carries them out, different rules apply, ranging from legal restrictions on intelligence organizations to regulatory and ethical frameworks that govern research.

The variety of entities and actors involved highlights how these studies—particularly those that directly involve members or sympathizers of violent groups—complicate traditional categories of research and raise complex ethical questions. Involvement of academic researchers in this field has not been without controversy (3). In recent years its management, particularly the effectiveness of systems to protect human subjects of research through Institutional Review Boards (IRBs), has been called into question (4).

IRBs and Terrorism

When research involves human subjects, regulations require subjects to be protected, forcing investigators and IRBs to address concerns about individual risk and benefit. Questions of justice and beneficence—core elements of the ethical principles underlying the contemporary system for protecting subjects—can be problematic where one party in a conflict is studying another. Although not always the case, research funded by a government (or other entity) on a terrorist opponent is often counter to the group or its members' interest. This differs from most research considered by IRBs that lacks this adversarial character.

Although research can benefit participants, studies on terrorism can do so in distinctive ways, including disseminating groups' messages or otherwise advancing their agendas. That potential for benefit has led some academics to question whether such research conflicts with laws forbidding material support to terrorist groups (5), which further complicates research management and review.

Risk to subjects also arises in different ways, coming in many cases from actions others might take if subjects' participation became known publicly. This creates additional tension about requirements for disclosure in recruitment, sensitivities around

A recent court case calls into question the ability to protect human subjects in research on terrorism and conflict.

deception, and concerns about data protection. Rarely are beneficence and justice, as traditionally construed, directly addressed.

These challenges have proven difficult for IRBs, with panels reaching very different decisions on similar studies, "standard rules" applied in ways that increase rather than decrease risk (e.g., retaining signed consent forms from subjects), and cases where researchers are unable to navigate approval processes (4, 6). Unlike most research, denying approval of a project on terrorism may only serve to shift who interacts with the subjects—as collection of such data is well within the mission of numerous government agencies—and, therefore, not actually result in the protective outcome desired (7).

Although the central driver for protecting subjects comes from ethical or legal regulation, it is also the case that it would be impossible to gather many types of primary data on terrorism without such protections. As a result, although the protections sometimes cover individuals who have been involved in heinous acts, doing so advances the societal interest in better understanding terrorism—a balance struck previously regarding research on other criminal and violent behavior.

Practical Protection to Enable Research

The authors are all members of the IRB of the RAND Corporation, an organization that has carried out numerous studies on terrorism. Our IRB has had to wrestle with these questions on studies including surveys in conflict regions, individual interviews, and anthropological fieldwork (7). Because risk in these studies is almost always driven by the prospect that the identity of participants may become widely known, strategies developed by our IRB and researchers focus on protecting confidentiality, when subjects cannot be anonymous during collection or in stored data. Measures include immediate separation of identifiers from collected data; selective recording of information to reduce potential for identifiability by inference; procedural controls, including rapid comingling of data to make linking responses to an individual more difficult; and technical controls like encryption to protect data in transit and storage. The need to make it possible

United States v. Moloney

This 2012 decision by the U.S. Circuit Court of Appeals for the First Circuit calls into question one critical tool for protecting research subjects—the researchers' assurance of confidentiality (8). Between 2001 and 2006, researchers collected oral history interviews from members of the Provisional Irish Republican Army (PIRA) promising confidentiality until after the subjects' deaths. Emphasizing the international nature of terrorism, in 2011, the British government, via the mutual legal assistance treaty between the countries, requested subpoenas (through the U.S. commissioner of that treaty) be sent as part of an investigation into the murder of Jean McConville, who was allegedly abducted and killed by the PIRA in 1972 (8). Subpoenas were issued for the tapes of one interviewee who was still alive, Dolours Price, which would break the assurance of confidentiality made to her. The researchers involved resisted and lost in the trial court and the court of appeals.

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for individuals to deny participation if they believe they need to do so is similar in many ways to what is required in studies involving incarcerated subjects—e.g., maintaining the ability of a prisoner to deny to his cell mate or guards that he spoke to an outsider doing research on incarceration. Signed consent forms have almost never been required, the IRB instead focusing on ensuring that the process of consent provides potential participants with information needed to make informed decisions. Furthermore, strategies used by our IRB and researchers during analysis of data sets collected by others generally rely on anonymization of data to minimize risk to individuals included.

Fragile Protection?

Our approach to human subjects protection emphasizing confidentiality and anonymization has provided a way to carry out research on these topics in a manner we judge to be in accord with regulatory and ethical requirements. Yet recent legal developments demonstrate the fragility of that strategy and risk the IRB approvability of an important subset of terrorism research [see *United States v. Moloney* sidebar (8)].

Although there are a number of complexities associated with the *Moloney* case, including that, as oral history, the project was apparently viewed as not requiring review by the Boston College IRB (9, 10), the appellate court's statements concerning confidentiality assurances are troubling. The researchers had argued that compelling disclosures would impede their ability to conduct research, activity protected by the First Amendment to the U.S. Constitution. The court rejected the argument, noting the government's strong interest in compelling disclosure of evidence in a criminal case, and ordered disclosure. The researchers have petitioned the U.S. Supreme Court to review the appellate court's decision, but review is rarely granted. Further complicating the case, the subject involved has since died, and the status of the appeals is yet undecided. Accordingly, this precedent may well stand.

If the government interest in enforcing the subpoenas in *Moloney*—where the data relate to actions decades ago—was deemed high enough to override confidentiality, the government interest in obtaining information concerning more recent incidents of violence would be at least as strong. Such a precedent calls into question the use of confidentiality assurances to reduce risk and thus facilitate IRB approval. It therefore is likely to chill research, at least any research where subjects are not anonymous to the researcher.

An Ethical Path Forward?

Government has an interest in pursuing evidence to solve crimes, and the murder at issue in *Moloney*—reportedly PIRA suspected McConville of being an informant—is a terrible one. However, the ruling neglects another rationale for protecting these subjects: societal interest in enabling in-depth research on terrorism.

This intersection of research and information about crime is not unique to terrorism research. In some areas, society has decided that research materials should be shielded from subpoena to enable research on sensitive issues or criminal behavior. One mechanism to gain that protection is through Certificates of Confidentiality (CoCs), available from the U.S. National Institutes of Health for work in its mission areas, that protect data from compelled disclosure (11, 12). Research supported by the U.S. Department of Justice (DoJ) on crime and criminality is given strong protection from subpoena by statute (13).

If the work of the researchers in *Moloney* had been funded by the DoJ, rather than the university (8), such statutory protection would have protected the data. But that organization represents only a portion of the terrorism research funded in recent years. In the absence of (unlikely) legislative changes that would broaden statutory protection, obtaining CoCs would be advisable for terrorism research involving nonanonymous subjects. Although CoCs may not be available for all such research (11), and the scope of the protections they actually provide from subpoena remains uncertain (14), the protection they provide would appear the best option available to enable terrorism research where subjects are known to the researchers.

Even if absolute confidentiality could be assured, if disclosure of research data could prevent an attack, wouldn't there be an ethical obligation to disclose? In other fields, if researchers uncover evidence of child or elder abuse, they may feel ethically or legally compelled to report it to prevent imminent harm. The conflict between reporting and assurances of confidentiality is resolved by disclosure during consent, making clear the consequences for revealing such information. Similar approaches have been applied in terrorism research. More consistency across IRBs—both in use of CoCs and inclusion in consent when confidentiality will be broken—could be a step in establishing a consensus balance between the pursuit of justice for past crimes and research intended to help prevent future ones.

Important changes in U.S. regulations underlying protection of human subjects are

under consideration (2). Because of unique issues in terrorism and conflict research, the proposed changes largely do not address the challenges discussed here. For example, although seeking to improve protection of identifiable data, the proposed changes focus on collected data (versus risk of disclosure during collection that is often prominent in conflict zones) and are silent on legally mandated disclosures after collection. The revision process is ongoing, meaning a window for addressing these concerns is still open.

Even if changes in the legal landscape signify that much research on terrorism or conflict involving human subjects becomes difficult or impossible, data will still be collected from such individuals by law enforcement and intelligence organizations charged with fighting violent groups. The effect would be to limit the production of generalizable knowledge on these groups, behaviors, and activities, in contrast to knowledge produced from the particular perspectives and supporting the specific operational demands of law enforcement or intelligence. Such a shift would keep the information from entering the academic literature, limiting the ability of the academy, and the public, to contribute to policy debate on a critical topic in an informed way. In assessing proposed revisions to regulations for protecting human subjects (2)—and instruments that support their implementation—issues and risks inherent must be addressed in research on terrorism, conflict, and, by extension, crime and criminality, where competing societal interests affect the ability to make and maintain promises of confidentiality.

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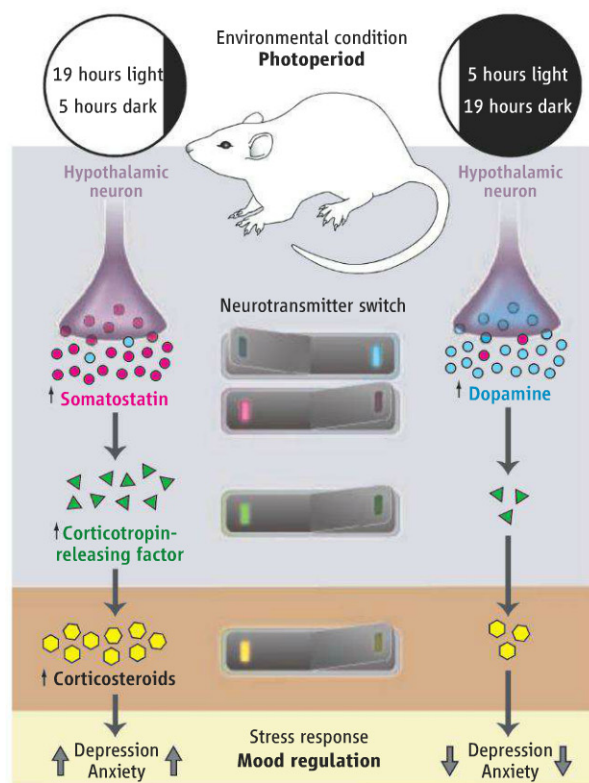
NEUROSCIENCE

Plasticity in the Neurotransmitter Repertoire

Susan J. Birren and Eve Marder

In the earliest days of neuroscience, it was thought that a neuron made and released only a single chemical, known as a neurotransmitter, to send a signal across a synapse to an adjacent neuron (1). At the same time, it was deeply mysterious why so many signaling molecules were used in nervous systems. Subsequently, it became clear that many, if not most, neurons (including those in mammals) make and release two or more neurotransmitters including small-molecules and neuropeptides (2–4). As the list of these potential cotransmitters and their receptors has increased, we are faced with understanding the functional relevance of this embarrassment of riches for neural circuits and behavior. Neurotransmitters and neuromodulators (substances often released with small-molecule neurotransmitters) can elicit a variety of different actions on their neuron targets, including directly opening ion channels or acting through signal transduction pathways to alter neuronal excitability or synaptic transmission. Thus, characterizing the mixture of cotransmitters released by a neuron is important for understanding how neuronal circuits operate. The mechanisms that change the profile of neurotransmitter release provide opportunities for plastic changes in circuit function, and consequently in organism behavior. On page 449 of this issue, Dulcis *et al.* (5) report changes in the neurotransmitter profile of neurons that underlie photoperiod-triggered changes in animal behavior. The findings argue that neurotransmitter switching is a new mechanism for neuroplasticity (6) in adult nervous systems.

Early studies using cultured developing neurons showed that individual neurons can switch their transmitter phenotype (7, 8). For example, peripheral sympathetic neurons that normally release norepinephrine as their neurotransmitter can undergo a developmental switch to acetylcholine. A role for this switch was shown for sympathetic neurons innervating rat sweat glands. These neurons release acetylcholine when innervating sweat glands, whereas they secrete norepinephrine when innervating other organs, including the



Neurotransmitter switch. During long days, there is a shift in the neurotransmitters released by hypothalamic neurons in the adult rat brain from dopamine to somatostatin, whereas the opposite shift is seen during short days. Increased dopamine signaling during short days results in decreased release of CRF from target neurons, and consequently less CRF and corticosteroids in the plasma. These conditions are associated with a decrease in stress behaviors in nocturnal rodents. The converse is seen with decreased dopamine signaling during long-day conditions. In humans and in other diurnal animals, short days are more stressful and are associated with depression.

heart (9). Subsequent work has studied the molecular pathways underlying these developmental switches (10, 11) and has demonstrated activity-dependent transmitter plasticity in adult rodent brains (12). This type of regulation became even more intriguing when it was found that the production of new neurotransmitters by neurons can induce new behaviors such as pigmentation changes in amphibian larvae (6, 13).

The work by Dulcis *et al.* is remarkable in that it ties these well-characterized phenomena to plasticity in the mammalian response to the light-dark cycle. Specifically, the exposure of adult rats to light was altered by

A switch in the type of neurotransmitter released in the brain underlies changes in mammalian behavior associated with day-night cycles.

keeping the animals in photoperiod chambers for a week on either long-day (19 hours of light and 5 hours of dark) or short-day (5 hours of light and 19 hours of dark) cycles. The number of dopamine-releasing neurons in several hypothalamic nuclei (clusters of neurons) increased with short-day cycles and decreased with long-day cycles, whereas the inverse was seen with somatostatin. Dopamine is a neurotransmitter whose functions in the brain include modulating cognition, motivation, mood, memory, and learning; somatostatin is a peptide neuromodulator that is widely expressed in the nervous system and may be involved in the regulation of stress responses. The number of dopamine receptors on target neurons in the brain increased and decreased homeostatically, likely to ensure that the changes in the cotransmission of dopamine and somatostatin would result in functional outcomes. Strikingly, the animals' behavior was also modified with short-day cycles, as seen in two assays thought to indicate mood, anxiety, and

depression. Changes in light-dark cycle have profound effects on human mood and behavior as well, contributing to a variety of disorders such as seasonal affective disorder. Thus, there is now a potential mechanistic link among mood, photoperiod, and neurotransmitter plasticity that mirrors associations observed in humans among seasonal affective disorder, photoperiod, and dopamine signaling (14).

Supporting this link are measurements of corticotropin-releasing factor (CRF) secretion by adult rat hypothalamic neurons in response to different photoperiods. Dulcis *et al.* noted that the change in the ratio of dopa-

minergic signaling to somatostatin signaling correlated with changes in the amount of CRF and corticosteroid in the plasma (see the figure). CRF is released by neurons in the mammalian brain that are targets of dopamine and somatostatin. This triggers a cascade of events that raises the concentration of circulating corticosteroids. These steroids have a wide range of physiological effects and have been implicated in stress and depression (15). The findings suggest that the transmitter switch potentially couples photoperiod and mood regulation. The ability of neurons to switch their neurotransmitter repertoire has been known for 40 years; the study of Dulcis *et al.* demonstrates

the use of this mechanism to control adult behavior in response to sensory variation.

Although the work by Dulcis *et al.* was carried out in nocturnal rodents for which long-day photoperiods are stressful, it is possible to imagine broader implications of this work for human behavior in which short-day photoperiods are stressful. Admittedly, the mechanistic details in humans may be different. Nonetheless, given the ubiquitous ability of neurons to release multiple neurotransmitters and the demonstrated capacity for plasticity, it is critical to consider the potential role of transmitter plasticity in understanding the human brain in health and disease.

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BEHAVIOR

Animal Conformists

Frans B. M. de Waal

More than 60 years ago, Kinji Imanishi speculated that if animals can learn from each other, they will inevitably develop different behaviors in different groups, resulting in “cultural” variation within the same species (1, 2). It was a simple proposal, but so far ahead of its time that few Western scientists paid attention. Only in the past few decades has animal culture taken off as a topic of research. Two reports in this issue give hints of the adaptive value of animal culture. On page 485, Allen *et al.* (3) show that the best explanation for the spreading of a new hunting technique among humpback whales (*Megaptera novaeangliae*) is that they learn from each other, and on page 483, van de Waal *et al.* (4) report an innovative field experiment that manipulated the food preferences of wild vervet monkeys (*Chlorocebus aethiops*) to give them an opportunity for social learning.

The early debate about animal culture focused on the mechanism of behavioral transmission. Do animals learn from each other in the same way as humans do? If they copy the behavior of others, does this reflect “true” imitation, that is, do they understand the other’s goals and methods? Apes were said to lack imitative capacities because they failed to imitate human models (5). But of course, human models belong to a different species. We now know that apes learn from each other in ways that meet all the requirements of true imitation (6–8).



Cultural feeding behaviors. (A) A whale whacks the water surface with its tail, a behavior that may increase the effectiveness of feeding on schools of small fish. Allen *et al.* show that this behavior was spread through social learning. (B) Van de Waal *et al.* report that vervet monkeys acquire food preferences from their mothers. In the image, Dublin, an adult male vervet monkey, eats pink corn with juveniles of his group. The monkeys do not touch the previously distasteful blue corn, even though in this phase of testing, both colors are palatable (4).



With that issue behind us, animal culture studies have begun to focus less on the transmission process and more on the strength of animal conformist tendencies and their effect on survival. From the domain of learning, researchers are shifting to that of outcomes and adaptive significance. The reports by Allen *et al.* and van de Waal *et al.* nicely illustrate this new focus.

Allen *et al.* report on an impressive large-scale project, covering 73,790 sightings of individually identified humpback whales in a particular area in the Gulf of Maine over the course of 27 years. Observations were made at the spawning ground of important prey that occurs in large quantities, the sand lance (a 15- to 20-cm fish). The whales used regular bubble feeding, in which they pro-

duce air bubbles under the surface to drive the fish together so that they can swallow thousands in a single gulp. However, in 1980, a whale was seen to whack the water surface with his fluke to create a loud noise or disturbance that may have clumped the prey even more. Over the ensuing years, this lobtail technique (see the figure, panel A) became increasingly common in the whale population.

The authors used a network-based diffusion analysis to determine whether being associated with whales that perform the lobtail technique helped naïve whales to adopt the same behavior. Independent of how much time the whales spent at the research site, they were more affected by exposure to skilled social partners than by any other

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CREDIT: (A) JENNIFER ALLEN AND THE OCEAN ALLIANCE; (B) ERICA VAN DE WAAL

potential factor. With a species that is not particularly suitable for controlled experiments, this is as close as we may ever get to proving that habits spread socially. Alternative hypotheses, such as genetic determination of the pattern or individual learning, were unsupported by the analysis.

Because of the neophobia of wild animals, field experiments are hard to conduct. Van de Waal *et al.* succeeded in inducing cultural transmission among wild vervet monkeys using a very elegant, controlled paradigm. They show that groups of wild monkeys can learn to distinguish between differently colored corn furnished by the experimenters. One color was palatable, the other highly distasteful. The two colors were counterbalanced across groups, so that corn of one color was palatable in some groups yet distasteful in others, and vice versa. All groups developed an exclusive preference for the untreated corn.

This by itself is just an example of individual learning, but then the investigators waited for new infants to be born in the groups and for new male immigrants to

arrive from neighboring groups. When the researchers gave both groups of monkeys access to palatable corn of both colors, the original residents, showing great conservatism, stuck to their acquired preference (see the figure, panel B). Twenty-six of the 27 newborn infants also ate only the locally preferred food; the one exception was the infant of a female so low in rank that she had been forced to taste the alternative corn in her offspring's presence. The behavior of all infants supported the thesis that food preferences are acquired from mothers and not through individual exploration.

Male immigrants, too, adopted the local color preference, in most cases despite their arrival from groups that had developed the opposite preference. The only exception was an immigrant male who immediately achieved the top rank position in his new group, and who as a result may have been less sensitive to the behavior of the locals.

The two studies (3, 4) show that cultural learning in animals is not a mere luxury that they occasionally engage in, but a common and widespread mechanism of behav-

ioral acquisition. Conformism among animals—that is, the tendency to prefer behavioral options that are common in the local population despite familiarity with or the presence of alternative options—has been indicated by experiments on apes (6) and monkeys (9) and documented in behavioral field research (10). Its confirmation in field experiments suggests that the mechanism is robust, with a great potential to affect survival success.

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ATMOSPHERE

Irreversible Does Not Mean Unavoidable

H. Damon Matthews¹ and Susan Solomon²

Understanding how decreases in CO₂ emissions would affect global temperatures has been hampered in recent years by confusion regarding issues of committed warming and irreversibility. The notion that there will be additional future warming or “warming in the pipeline” if the atmospheric concentrations of carbon dioxide were to remain fixed at current levels (1) has been misinterpreted to mean that the rate of increase in Earth's global temperature is inevitable, regardless of how much or how quickly emissions decrease (2–4). Further misunderstanding may stem from recent studies showing that the warming that has already occurred as a result of past anthropogenic carbon dioxide increases is irreversible on a time scale of at least 1000 years (5, 6).

But irreversibility of past changes does not mean that further warming is unavoidable.

The climate responds to increases in atmospheric CO₂ concentrations by warming, but this warming is slowed by the long time scale of heat storage in the ocean, which represents the physical climate inertia. There would indeed be unrealized warming associated with current CO₂ concentrations, but only if they were held fixed at current levels (1). If emissions decrease enough, the CO₂ level in the atmosphere can also decrease. This potential for atmospheric CO₂ to decrease over time results from inertia in the carbon cycle associated with the slow uptake of anthropogenic CO₂ by the ocean. This carbon cycle inertia affects temperature in the opposite direction from the physical climate inertia and is of approximately the same magnitude (2, 6).

Because of these equal and opposing effects of physical climate inertia and carbon cycle inertia, there is almost no delayed warming from past CO₂ emissions. If emis-

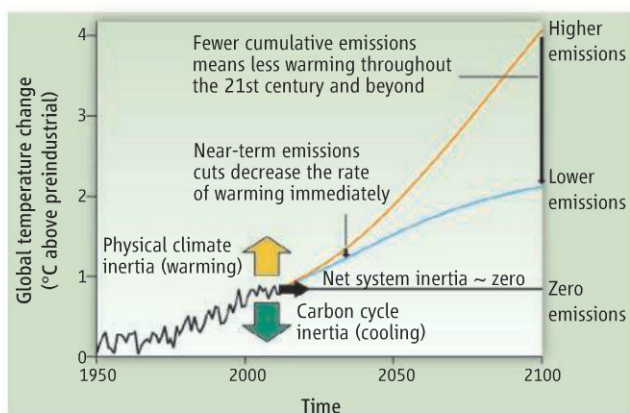
Carbon dioxide emissions cuts implemented today would affect the rate of future global warming immediately.

sions were to cease abruptly, global average temperatures would remain roughly constant for many centuries, but they would not increase very much, if at all. Similarly, if emissions were to decrease, temperatures would increase less than they otherwise would have (see the first figure).

Thus, although the CO₂-induced warming already present on our planet—the cumulative result of past emissions—is irreversible, any further increase in CO₂-induced warming is entirely the result of current CO₂ emissions. Warming at the end of this century and beyond will depend on the cumulative emissions we emit between now and then. But future warming is not unavoidable: CO₂ emissions reductions would lead to an immediate decrease in the rate of global warming.

Why, then, are many different near-term projections of CO₂-induced warming very similar? These modeled estimates are similar because even socioeconomic scenarios that produce very different cumulative emissions by the end of this century are not very differ-

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How the climate system responds. The climate response to CO₂ emissions is influenced by both physical climate and carbon cycle inertia, with the result that the net system inertia is close to zero. Therefore, future climate warming depends only on current and future CO₂ emissions, and the rate of warming will respond immediately to CO₂ emissions cuts. The illustrative future scenarios shown here are from SRES scenarios B1 (blue line) and A1FI (orange line). Idealized future warming is calculated as a linear function of cumulative CO₂ emissions. Observed historical temperatures are shown in black.

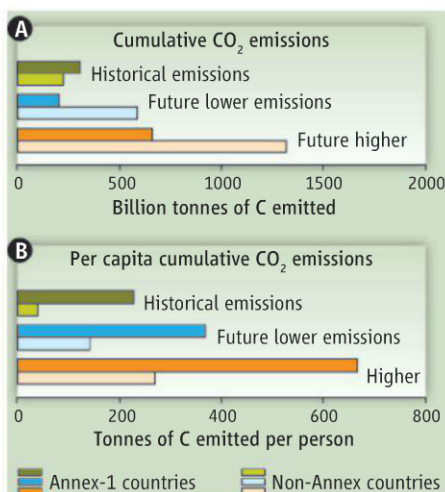
ent over the next two decades (figs. S1 and S2). The climate system physics implies that further increases in warming could in principle be stopped immediately, but human systems have longer time scales. Carbon-emitting infrastructure is designed to benefit humankind for many decades; each year's additional infrastructure implies added stock intended to last and emit CO₂ for many decades. It is this dependence on CO₂-emitting technology that generates a commitment to current and near-future emissions (7). Cleaner alternatives are being developed and carbon capture and storage technologies are being tested, but technological development and diffusion are subject to substantial inertia (8). Societal inertia, rather than the inertia of the climate system, is thus the critical challenge if we wish to begin to decrease the rate of CO₂-induced global warming in the near future.

The strong dependence of future warming on future cumulative carbon emissions implies that there is a quantifiable cumulative amount of CO₂ emissions that we must not exceed if we wish to keep global temperature below 2°C above preindustrial temperatures. Several recent analyses have suggested that total CO₂ emissions of ~1000 Pg C (~3700 Pg CO₂; 1 Pg = 10¹⁵ g) would give us about even odds of meeting the 2°C target (9–12). To meet such a target given historical emissions would mean that the world has roughly half of the allowable emissions budget remaining. This is equivalent to 50 years of emissions at current levels and carries the implication that the longer we delay before beginning to decrease emissions, the faster

the rate of decrease must be to stay within this total allowable budget (13).

Emissions differ widely between countries, particularly between those in the developed and developing world (14). Cumulative carbon emissions from the developed world currently exceed those from developing countries, but rapid economic growth in emerging economies is expected to reverse this pattern within a few decades (fig. S2). Nonetheless, per capita cumulative emissions from developed countries are expected to remain far higher than those from developing nations throughout the 21st century (see the second figure).

If technological investments and innovation increase the availability of reduced-carbon sources of energy that are competitive in price, development can continue to improve the lives of people in emerging economies without driving global climate change to increasingly dangerous levels. If reduced-carbon energy sources are not advanced rapidly, a great deal of carbon-intensive infrastructure



Development and emissions. Cumulative emissions from developed countries (Annex-1) currently exceed those from developing countries (non-Annex). This pattern is expected to reverse for future emissions scenarios (A), but per-capita cumulative emissions from developed countries are expected to remain much higher than those from developing countries (B). Historical emissions until 2012 are shown in green; future cumulative and per capita cumulative emissions are calculated at year 2100 for SRES B1 (blue) and A1FI (orange) emissions scenarios.

is likely to be put in place in the developing world, implying a large and ongoing societal commitment to further global CO₂ emissions and consequent climate warming (7).

Given the irreversibility of CO₂-induced warming (5, 6), every increment of avoided temperature increase represents less warming that would otherwise persist for many centuries. Although emissions reductions cannot return global temperatures to pre-industrial levels, they do have the power to avert additional warming on the same time scale as the emissions reductions themselves. Climate warming tomorrow, this year, this decade, or this century is not predetermined by past CO₂ emissions; it is yet to be determined by future emissions. The climate benefits of emissions reductions would thus occur on the same time scale as the political decisions that lead to the reductions.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1236372/DC1
Supplementary Text
Figs. S1 and S2
References

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MOLECULAR BIOLOGY

A Circuitous Route to Noncoding RNA

Jeremy E. Wilusz and Phillip A. Sharp

Most genetic information is expressed as, and transacted by, proteins. Yet, less than 2% of the human genome actually codes for proteins, prompting a search for functions for the other 98% of the genome, once considered to be mostly “junk DNA.” Transcription is pervasive, however, and high-throughput sequencing has identified tens of thousands of distinct RNAs generated from the non-protein-coding portion of the genome (1). These so-called noncoding RNAs vary in length, but like protein-coding RNAs, appear to be linear molecules with 5' and 3' termini, reflecting the defined start and end points of RNA polymerase on the DNA template. But do all RNAs have to be linear?

Circular RNAs that have covalently linked ends are found in pathogens such as viroids (virus-like infectious particles), circular satellite viruses, and hepatitis delta virus—which causes severe liver disease in humans infected with hepatitis B and replicates by a rolling-circle-based mechanism (2). A handful of circular RNAs generated from eukaryotic genomes have also been identified (3, 4), but their role is unclear as they are generated by seemingly rare errors in RNA splicing. Because eukaryotes contain split genes, their precursor mRNAs (pre-mRNAs) must be modified such that noncoding introns are removed and protein-coding exons are joined together (see the figure). In these rare cases that generated circular RNAs, the splicing machinery failed to join the 3' end of one exon to the 5' end of the next and instead appeared to mis-splice by, for example, joining the two ends of a single exon together. High-throughput sequencing data combined with new computational algorithms (5–8) have now revealed thousands of circular RNAs in species ranging from humans to archaea.

Human fibroblasts alone have more than 25,000 circular RNAs (5). Derived from ~15% of actively transcribed genes, these circles contain mostly exonic sequences (usually between one and five exons). Large

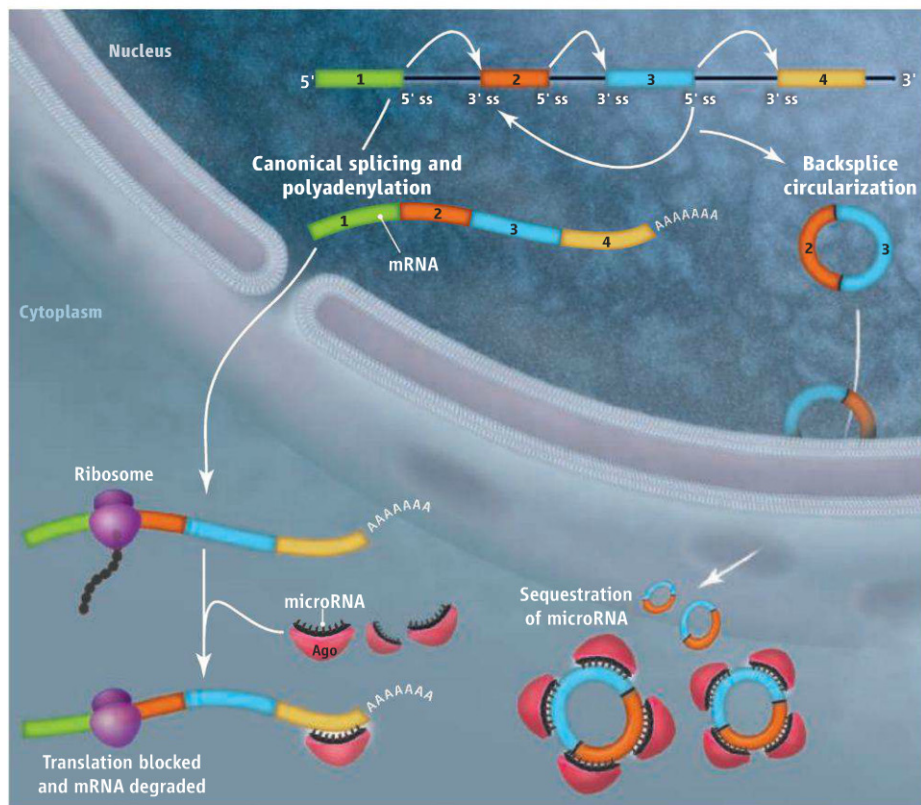
numbers of circular RNAs accumulate in the cytoplasm of cells, sometimes exceeding the abundance of the associated linear mRNA by a factor of 10 (5, 7). This is likely because circular RNAs are resistant to degradation by many cellular RNA decay machineries, which recognize the ends of linear RNAs.

For a subset of circular RNAs, the circularization signals appear to be evolutionarily conserved, as some were detected in both human and mouse (5, 6). The splicing machinery is very likely involved in their biogenesis as canonical splicing signals generally immediately flank their sequences. However, the exact mechanism by which the splicing machinery selects particular regions to circularize is still unclear. The presence of inverted repeats in the surround-

A class of circular RNAs that regulates microRNAs is abundant in mammalian cells.

ing introns and/or exon skipping events may play a role (3, 5, 9).

One particular circular RNA found predominantly in human and mouse brain is ~1500 nucleotides in length and, surprisingly, contains more than 70 evolutionarily conserved binding sites for the microRNA miR-7 (6, 9). MicroRNAs are ~21-nucleotide RNAs that function by base pairing to mRNAs and repressing protein production and/or causing mRNA degradation (10). However, a microRNA can bind to any transcript that contains a complementary sequence and, indeed, this circular RNA may efficiently bind ~20,000 miR-7 microRNAs per cell (6), thereby preventing miR-7 from binding other RNAs. Consistent with a “sponging” model (11), decreasing expression of this



Circular RNAs sequester microRNAs. Noncoding introns [flanked by splice sites (ss)] are removed from pre-mRNA by the splicing machinery and a poly(A) tail is added to prevent mRNA degradation (left). Linear mRNA is then translated by ribosomes unless it becomes bound by microRNAs that are associated with argonaute (Ago) protein. The latter interaction inhibits translation and/or promotes mRNA degradation. The splicing machinery can also “backsplice” and generate circular RNAs whose 5' and 3' ends are covalently linked (right). Large numbers of these circular RNAs sequester microRNAs from binding mRNA targets.

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circular RNA in cells caused mRNAs containing miR-7 binding sites to also decrease in number, indicative of increased binding of miR-7 to these mRNAs leading to their degradation (6, 9).

Additional circular RNAs may function similarly to regulate the activity of other microRNAs because, in general, microRNA binding sites are abundant in circular RNAs (6). For example, in the mouse, sex-determining region Y (*Sry*) plays a key role in testes development and generates a circular RNA that contains 16 miR-138 binding sites (9). Circular RNAs thus join a growing class of naturally occurring microRNA “sponges,” which includes competing endogenous RNAs and pseudogene (nonfunctional gene) RNAs (12). Compared to competing endogenous RNAs and pseudogene RNAs, however, circular RNAs are likely much more potent microRNA sponges as they are expressed in greater amounts and contain many more microRNA binding sites. Furthermore,

unlike competing endogenous RNAs, circular RNAs appear to be resistant to RNA degradation triggered by microRNAs and thus could have long half-lives (9).

Beyond regulating microRNAs, circular RNAs may bind and sequester RNA-binding proteins or even base pair with RNAs besides microRNAs, resulting in the formation of large RNA-protein complexes. Other circular RNAs may produce proteins, given that synthetic circular RNAs can be efficiently translated (13). As even linear mRNAs are thought to circularize during translation through protein-protein interactions between factors binding the 5' and 3' ends of the mRNA, RNA circularization, whether by direct covalent bonds or noncovalent means such as protein bridging or Watson-Crick base pairing, may be much more common than is currently appreciated. The discovery of such a large class of previously unknown RNAs also raises the question of what other RNAs might have been missed. Considering that RNA

structural elements, such as triple helices, efficiently prevent degradation from RNA termini (14), it is becoming increasingly clear that the cell uses a myriad of distinct ways to process and stabilize RNA molecules.

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CHEMISTRY

Are Gold Clusters in RF Fields Hot or Not?

Hong Koo Kim,¹ George W. Hanson,² David A. Geller³

Nanoparticles are finding increasing applications in diagnostics, imaging, and therapeutics in biology and medicine. Gold nanoparticles (Au NPs) have received a great deal of interest because of their distinctive optical, electronic, and molecular-recognition properties, as well as biocompatibility. One promising yet ambitious application for Au NPs is noncontact hyperthermia of cancer. By attaching tailor-made ligands, Au NPs can recognize and lock onto receptors on the surface of tumor cells. Under illumination by light, Au NPs can absorb radiation energy and selectively heat and destroy tumor volumes without collateral damage to neighboring healthy tissue. This type of noninvasive, specific, active targeting with Au NPs is highly desirable from a clinical perspective, but its use in cancer intervention has yet to be demonstrated.

The use of radio-frequency (RF) waves instead of light for heating Au NPs is appealing because of the larger penetration depth of RF waves in tissue, which would increase the tissue volumes that can be interrogated. A number of experimental studies have reported increased tumor cell destruction in the presence of Au NPs. One capacitive RF apparatus, in which a 13.56-MHz RF field is applied across two parallel plate electrodes (1), can produce marked heating (1–3). Moran *et al.* hypothesized that the increased heat dissipation in Au NP solutions was caused by increased Joule (resistive) heating in Au NPs (3). By implicitly assuming that the applied electric field penetrates through the Au NP without attenuation (i.e., no shielding effect of free electrons in the metal), a phenomenal amount of power dissipation was calculated for Au NPs and was used to explain the observed solution heating.

This type of Joule heating of Au NPs by RF fields has been disputed on both experimental and theoretical grounds (4, 5). Li *et al.* showed that the heating of Au NP solutions arose from the background ionic solution, not the Au NPs themselves. After separating out

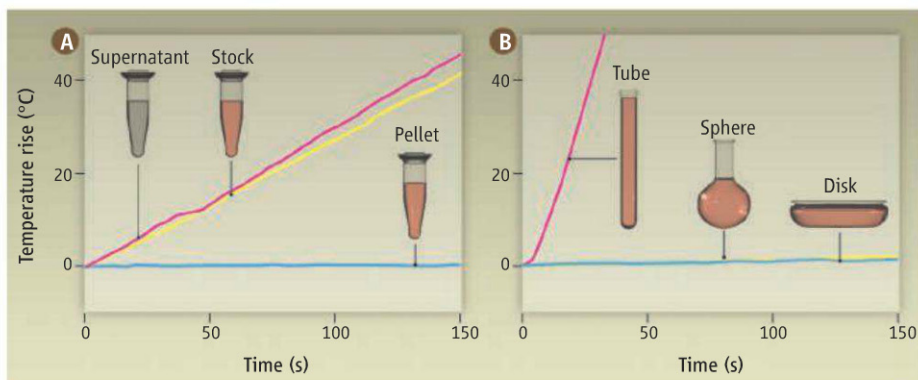
Heating of gold nanoparticles by radio-frequency waves, which is of interest for clinical applications, can occur if the nanoparticles are made magnetic.

Au NPs from the solution via centrifugation and resuspending them in deionized water, negligible RF absorption was observed for a variety of Au NPs ranging in size from 5 to 200 nm. These findings contradict previous conclusions about the heating rate dependence on particle size and concentration and disprove the hypothesis that Au NPs directly absorb RF energy.

Unlike the case at optical frequencies, where a photothermal effect can arise from electromagnetic field penetration into the particle, Au NPs respond differently to RF fields. Given the low oscillation frequency of RF energy, free electrons in metals respond essentially instantaneously and their drift cancels the incident electric field. This shielding effect renders Au NPs unheatable by RF electric fields; any observed heating is Joule heating of the ionic solvent (6).

McCoy *et al.* now report RF heating of gold cluster solutions tested in an inductively coupled RF apparatus—that is, inside a coil where the magnetic field dominates over the electric field (7). First, *p*-mercaptobenzoic acid (pMBA) was used to create a fixed-size crystalline nanocluster, Au₁₀₂(pMBA)₄₄.

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Rethinking RF heating of gold nanoparticle solutions. (A) Absorption of RF energy by solutions containing gold nanoparticles is caused by Joule heating of the background ionic solution (supernatant), not by gold nanoparticles (pellet). Sample volume, 0.5 ml. (B) Joule heating further depends on the shape of media confinement. A thin tube (0.65 ml) oriented parallel to the electric field heats faster than a sphere (1 ml) or a disk (1 ml). RF power, 25 W; Au NP concentration, 6×10^{-3} weight %.

These Au nanoclusters (Au NCs) can be considered as closed-shell, nonmagnetic superatoms that are not susceptible to RF fields. McCoy *et al.* report that the Au NCs become paramagnetic when oxidized with KMnO_4 , so that they can be heated by magnetic fields, similar to the heating of magnetic NPs (such as iron oxide NPs) in oscillating magnetic fields. In this type of heating, particles can rotate within the stationary solvent (Brownian relaxation) or magnetic dipoles can rotate within the particle (Néel relaxation).

McCoy *et al.* claim that other previously reported experiments might have contained unintentionally magnetic Au NPs in oscillating magnetic fields, partially accounting for the observed NP-dependent heating. Most previous reports of Au NP heating, however, used NPs with diameters of 5 nm or larger, and superatom stabilization and potential superatomic paramagnetism are unlikely in that size range.

The work by McCoy *et al.* represents an important technical advance, but more work is needed to understand the underlying mechanisms and to develop the technology for use in RF hyperthermia. Much of the heating observed with their Au NCs is modest and substantially less than that reported in previous work on RF heating of solutions and for magnetic heating of iron oxide NPs. Furthermore, considering that most tissues have an ionic background, Joule heating of the background media is expected to be an important factor that will affect the selectivity of RF heating.

Joule heating further depends on operating frequency (8) and the shape and geometry of media confinement. Recently, we reported excellent RF heating of ionic solutions at 13.56 MHz, but the shape of the container holding the NP solution (or any conductive

solution) played an important role (see the figure) (9). Hence, heating applications must be considered in light of container shape. For example, a long, thin object immersed in an RF field and oriented parallel to the electric field will allow greater field penetration into the interior relative to a low, flat object. The slight heating of solutions in a dish with electric field perpendicular to the dish surface

may be mainly a geometric effect, rather than a material property of the solution, because depolarization fields develop at dielectric interfaces in response to RF electric fields.

Similar effects are expected with the size and shape of the whole body or individual organs. At a smaller scale, capillaries and microvasculature would also have an important effect on the efficacy of local heating by RF fields. Considering the complex nature of radio-wave interactions in tissues *in vivo*, further studies are needed to develop optimal RF hyperthermia targeting strategies.

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GEOPHYSICS

Melting Earth's Core

Yingwei Fei

Studies of melting iron at extreme temperature and pressure provide a better understanding of the temperature of Earth's core.

Earth's predominantly iron core is under extreme pressures that range from 136 to 364 GPa (100 GPa $\sim$ 1 million atm). The core consists of an outer layer that is molten and a solid inner core. The temperature of the core region can be estimated if the melting temperature of iron under such extreme pressures can be determined. Pressures and temperatures corresponding to the conditions at the core can be generated in the laboratory by using two gem-quality single-crystal diamonds coupled with laser heating (see the figure). The challenge, however, is how to accurately determine the melting point under such extremes. On page 464 of this issue, Anzellini *et al.* (1) use fast *in situ* synchrotron x-ray diffraction to study melt-

ing in the laser-heated diamond-anvil cell. The accurate determination of the melting temperature of iron provides an important constraint on the core temperature, which is essential to understanding how the dynamic Earth works, including its heat budget, generation of its magnetic field, and the thermal evolution of the planet.

The primary experimental methods used to induce the melting of iron at extreme pressures are dynamic shock compression with a light-gas gun (2) and static compression in the diamond-anvil cell with laser heating (3, 4). Along the pressure-temperature path of shock-compression experiments, melting is detected as an abrupt decrease in the sound velocity with increasing shock pressure, but the shock-temperature determination is dependent on having an accurate thermodynamic model. By contrast, the temperature of melting in the laser-heated

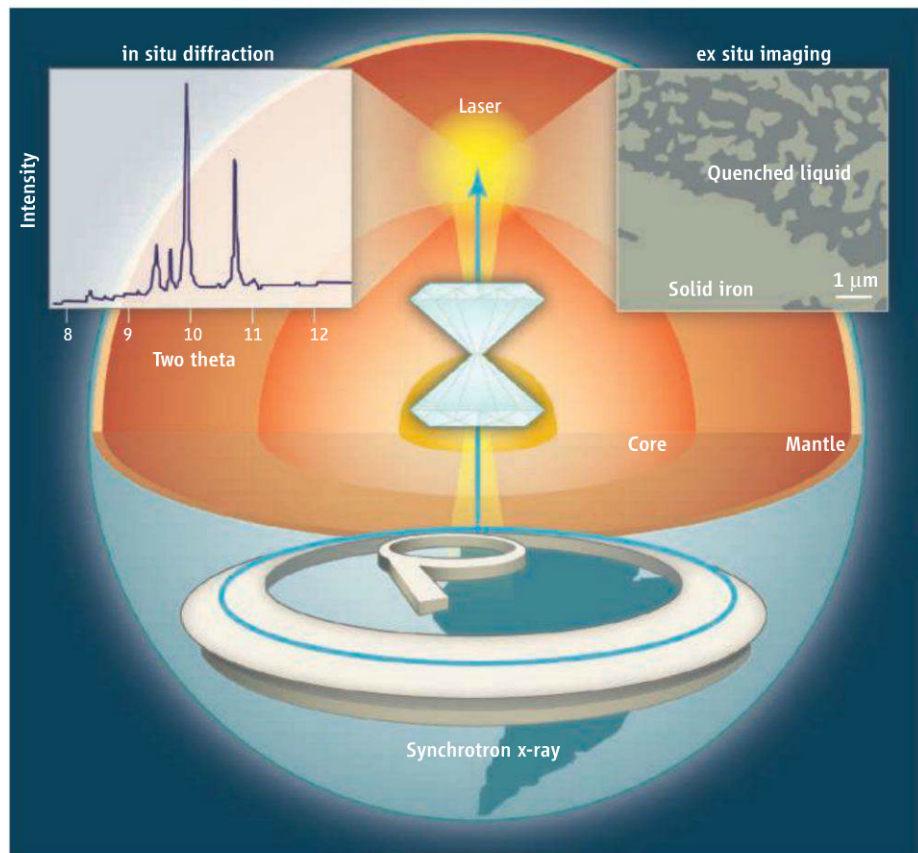
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diamond-anvil cell can be directly determined from the thermal radiation spectrum. In addition to considerable uncertainties in temperature determination from the dynamic and static techniques, the data also present an unresolved inconsistency, with the melting temperature of iron determined by shock compression lying appreciably higher than those from static measurements. The discrepancy in the estimated temperature of the inner core boundary (ICB) at 330 GPa is as large as 2000 K, which could influence the conclusions of dynamic and thermal evolution models. Theoretical melting curves also show wide variations and, thus, are not sufficient to resolve the discrepancy (5).

Melting experiments at core pressures are challenging. Determinations of the melting temperature of iron by the static method have been limited to pressures below 200 GPa, which corresponds to only the top one-quarter of the core (1, 3, 4, 6). Accurate determination of the melting curve requires not only reliable measurements of pressure and temperature, but also objective, reproducible melting criteria. Anzellini *et al.* provide a valuable addition to the melting criteria at high pressure by coupling static compression with fast in situ synchrotron x-ray diffraction measurements. The fast x-ray diffraction technique provides continuous monitoring of the changes in iron as a function of temperature. Using the onset of a diffuse x-ray diffraction ring alone as the melting criterion could lead to an overestimation of the melting temperature, but the combined observations of the increase in the lattice parameters and the appearance of the diffuse diffraction peak provide an unambiguous determination of the melting point (1). The melting temperatures determined with this new technique are appreciably higher than previous static-compression measurements (3), and slightly higher than previous in situ measurements at about 100 GPa (4), and are in agreement with the shock wave dynamic shock-compression measurements and recent quantum Monte Carlo simulations (7) and ab initio calculations (8).

The results reported by Anzellini *et al.* provide an independent measure toward resolving the discrepancy in the measured melting curve of iron at high pressure, but additional experiments based on other techniques are needed to ultimately address the issue. For instance, combining the fast in situ x-ray diffraction approach with ex situ chemical and textural characterizations of the quenched samples could be a powerful way to accurately determine the melting point and address the question of possible chemical contaminations or reactions during the



Probing extremes. Melting Earth's core in the laboratory by using laser-heating technique in the diamond-anvil cell. The laser-heated diamond-anvil cell is used to simulate the conditions at Earth's core. Melting of core materials can be detected by in situ synchrotron x-ray diffraction and textural examination of the quenched samples.

experiments. Such an approach is necessary for constraining the temperature of the core because the iron-dominated core also contains some nickel and about 10 weight % of light elements such as sulfur, silicon, oxygen, carbon, and hydrogen (9), which likely depress the melting curve of pure iron. Such a combination could also provide information about element partitioning between the coexisting solid and liquid phases. It is now possible to precisely mill a 20-μm laser-heated spot with a focused ion beam and, in turn, obtain high-resolution scanning electron microscope images of the heated spot and the chemical compositions of the coexisting phases with a highly efficient silicon drift detector, thus providing an unambiguous melting criterion. This integrated approach will yield a maximum amount of data from a core simulation experiment, resulting in a more complete understanding of the temperature and composition of Earth's core.

The work by Anzellini *et al.* represents an advance in melting temperature determination at ultrahigh pressure, with critical information about the structure and density of the solid phase right before melting. The ultimate

challenge for determining the temperature of the core is to obtain not only the melting temperatures of iron but also the light-element partitioning between liquid and solid iron at 330 GPa. This can only be achieved by further improving techniques that generate stable, simultaneous high pressures and temperatures corresponding to the conditions at the ICB, along with in situ x-ray diffraction and spectroscopic observations and ex situ characterizations of chemical composition and melting texture.

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IBI* SERIES WINNER

Root Cause Analysis for Young Engineers

Joe Immel

Engineers learn from mistakes. The mantra “fail often, fail fast” is sometimes heard in the research and development environment and describes a spirit of exciting exploration, investigation, and innovation. But the real world is a crucible for errors and flaws where the failure of functional designs can affect real people. There is always a human face to engineering design decisions, and the result of an unanticipated coincidence of events or conditions is the stuff of nightmares for engineers. Whether the result of failure is annoyance and embarrassment or catastrophe and tragedy, engineers use a systematic approach to discovering what went wrong.

The module “Root Cause Analysis: Methodologies and Case Studies” is a portion of the Senior Engineering Course at Technology High School. The root cause of a problem is the most fundamental issue and may be obscured by symptoms or secondary causes. The object of our module is to teach students a systematic approach to the analysis of complex problems involving engineering failures. Students at our school enjoy a project-based curriculum centered around integrated science and engineering courses. All courses, with the exception of mathematics and foreign language, are taught using a group-oriented, project-based approach. From freshman to senior year, students work in project groups that are assembled, organized, and reorganized in order to learn the same material taught elsewhere with a traditional lecture model. Every student is required to contribute in every possible role, and they all learn to bring something to the table in terms of talent and energy. For example, some students covet the role of leadership; others are less comfortable with it, yet each student must learn to take the reins and make a group work to produce the desired product. By the time our students are seniors, they can organize quickly and efficiently into teams to solve the problem at hand and accomplish the goal set forth by instructors.

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*IBI, Science Prize for Inquiry-Based Instruction; www.sciencemag.org/site/feature/data/prizes/inquiry/.

Root Cause Analysis, an IBI prize-winning module, requires students to use a systematic approach to analyze complex problems.



Problem definition. Students use sticky notes and large sheets of paper to construct fishbone diagrams or flow charts for root cause analysis.

discussions, students learn problem definition of adverse events as a method that steers investigations toward the root cause. Students are encouraged to ask “why” at least five times and to keep asking “who, what, when, where, how, etc.,” until they arrive at a root cause. We coach the students to find at least two root causes in the form of preexisting conditions and actions (1). The end game is to elicit recommendations for effective corrective and preventive actions (CAPA) outlined in class discussions at first and later in their executive summaries. Students are assured that their opinion is

The aim of the module is to provide students tools for the analysis of adverse events in engineering and to allow them to further develop and refine the methods themselves. Our engineering program spans 4 years, beginning in the freshman year with projects to introduce students to our research and development (R&D) and robotics shop through the senior year in which students develop their senior projects (their magnum opus) during the second semester. The Root Cause Analysis module is currently composed of five case studies that are progressively more complex and difficult to assess. The basic introduction stresses problem definition. The initial case studies utilize commercially available documentaries (e.g., History Channel: “Engineering Disasters”) that are interrupted before the “punch lines” are revealed. Examples, such as the Titanic disaster and design flaws in Liberty ships, are presented by the instructor to model problem definitions.

The first case study is the sinking of submarine Thresher (SSN-593). Through discussions in groups and round-robin classroom

as good as anyone else’s provided they’ve done their homework with due diligence.

When students are adept at problem definition, they are introduced to analysis methods including Ishikawa fishbone diagrams and flow charts. A pre-prepared investigation into the interstate highway I-35W bridge collapse in Minneapolis (2) introduces the fishbone-style organization tool. Working in groups, students use photographs and a video of the failure (available in the public domain, online) to arrive at their conclusions and their recommendations (see the first photo). The executive summary of the investigation report issued by the National Transportation Safety Board is used to model the requirements for executive summaries that students produce for the class (3). A simpler event (4) is used to introduce flow charting. For both methods, extensive use of sticky notes is encouraged to demonstrate and organize ideas. (Everyone loves sticky notes—students have been known to cover entire kitchen walls with their sticky-note analyses.)

PHOTO CREDIT: JOE IMMEL

Students at this point have learned problem definition, analysis methodologies, criteria for CAPA recommendations, and executive summary format. In the fourth case study, they employ these tools to analyze the blowout in the Gulf of Mexico at the Macondo deep-water well site. Products required are a group presentation to the class of a graphical analysis (fishbone or flow chart) and their first executive summary (see the second photo).

In the first four case studies, students learn the tools for analysis and gain confidence in the methodology. The fifth case study is as close to “real life” as we can make it. For this case study (5), students are asked to attend class at night during a quiet time on campus to minimize distractions. They engage in an intensive, 4-hour exercise in which small groups are required to acquire information that is known only to one of six engineers involved in the real adverse event (the Columbia space shuttle disaster). Instructors confer before the exercise to “seed” groups of seniors with students most likely to be able to adopt the personality of the engineer involved such that they can play them successfully in reenacting a critical mission management team meeting. After the meeting, the scenario switches immediately to the day of the disaster, where the students, still maintaining their roles as expert engineers, must face the public (instructors,

About the author



Joe Immel teaches integrated science and engineering at Technology High School on the campus of Sonoma State University in California. Before moving into public education, he was an R&D engineer at Sutter Instrument Company and technical evangelist at Axon Instruments, Inc. He earned degrees in biology, including a B.A. at the University of California, Santa Barbara; an M.A. at the University of California, Los Angeles; and a Ph.D. at the University of California, Santa Barbara, under the aegis of Steven K. Fisher. His postdoctoral scholarship was at Stanford Medical School, in the Department of Ophthalmology with Michael F. Marmor and at the University of California, San Francisco, in the departments of Ophthalmology and Physiology, as a National Eye Institute (NIH) Fellow with Roy H. Steinberg. Joe is also vice president at Immel Resources LLC, a consulting firm for the pharmaceutical, biotechnology, and medical device industries, where he works with his wife, Barbara Kephart Immel (president).

parents, local media, professors, etc., playing the roles of the press) in a brutal mock press conference. This “trial by fire” is probably the most intense education our students have ever encountered. After a closing lecture, students are sent off to collaborate but individually to write their executive summaries, which are due the next day, in 12 to 15 hours.

Great emphasis is placed on sharing ideas and knowledge, especially since students are in possession of only the information from the individual engineers. This is a dicey situation insofar as authentic, individual work is required (executive summaries). However, communication is the “way of the world,” and it is encouraged in this course. For the final product of the fifth exercise (executive summary for the “Columbia” disaster), students are encouraged to self-organize into teams to share information and documents generated during the process. Contact information is exchanged to promote the overnight discussion through cell phones, e-mail, Facebook, and texting. As instructors, we encourage them to use every available means to share ideas and content. Working in small groups at the library or at private homes, our students develop amazing analyses and produce executive summaries that rival the best written by professionals.

The morning after “CAPA Night” (as the final exercise has become known) is an eye-opener for younger students.

This is usually the last day of the semester, and instructors are gentle with senior students. Still, from the opening of the school day to the last minute of the executive summary deadline, seniors, in various stages of torpor and stupor, trundle in to submit their executive summaries, which leaves younger students agog. Since the inception of this program, seniors have been very good about keeping the content of the exercise confidential. Even though younger students observe the aftermath (exhausted senior students) for several years, for each new senior class the scope and breadth of CAPA Night comes as a surprise.

Root cause analysis—CAPA investigations are challenging and intellectually invigorating. Although this module ends with a grueling, intensive exercise, the results of this course are exhilarating for the students. They eagerly apply lessons learned at every opportunity and report that these skills hold them in good stead even after leaving Tech High.

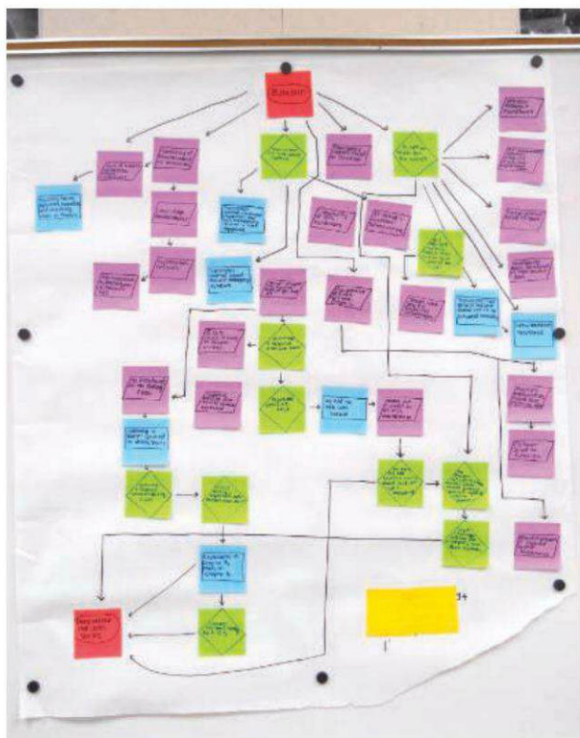
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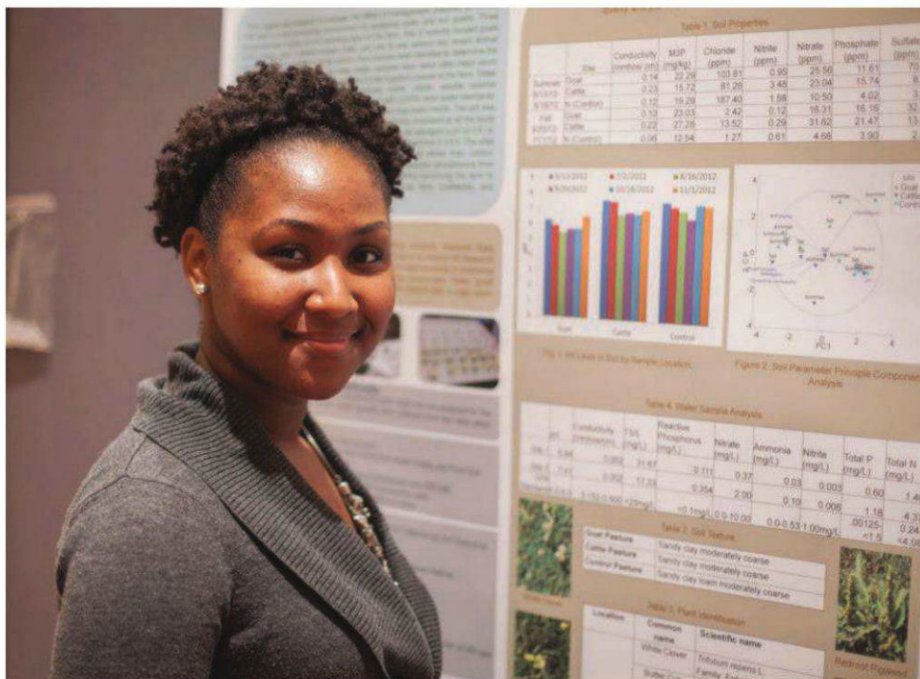
Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6131/444/DC1

10.1126/science.1229907



Root cause analysis diagram. A flow chart of the adverse event (blowout) at the Macondo well site that sank Deepwater Horizon in the Gulf of Mexico, 2010.



SCIENCE CAREERS

In a Competitive Market, New Scientists Seek Out Career Advice

Scientists may begin their studies with an eye on a tenure-track position, but these coveted jobs have been vanishing under a glut of science degrees and university budget cuts. As researchers found in a 2012 study in the journal *PLoS One*, jobs in academia become less attractive to U.S. doctoral candidates as they progress through their studies.

Resources to help early-career scientists find fulfilling jobs in an increasingly competitive academic market—and in less traditional fields—are thus in high demand. At conferences and online, experts from AAAS and other organizations are seeing a significant interest in constructive advice and improved tools for building better careers.

“We want to play a valuable role in helping emerging and new scientists to better understand science careers in a global context,” said Yolanda George, deputy director of AAAS Education and Human Resources, “as well as how to ‘tool-up,’ apply, persist, build networks, and to become leaders in all sectors of the scientific workforce.”

At the 28 February to 2 March Emerging Researchers National (ERN) conference,

hosted by AAAS and the National Science Foundation’s Division of Human Resource Development, more than 600 undergraduate and graduate students had a chance to test their research presentation skills before a diverse panel of judges. Representatives from 19 companies and 31 graduate schools also attended to recruit students for research fellowships and job opportunities.

Marcus Jones, an assistant professor in infectious disease and genomic medicine at the J. Craig Venter Institute in Rockville, Maryland, helped to judge the presentations. At conferences like ERN, students “get to learn an important skill: presenting their research and selling themselves,” Jones said. “Everyone should be able to give a 60-second speech to a millionaire about why they should fund your research.”

Ashli Allen, a senior at Fort Valley State University in Georgia, said that the ERN experience was useful and helped her focus on her plans to work toward a Ph.D. in physical therapy. “I did a lot of networking,” she said, “and met some amazing people who will be able to help me at the next level.”

Building success. Environmental sciences student Akida Ferguson of Delaware State University won first place for her presentation at the 2013 ERN conference.

Many of the ERN judges were undergraduate alumni of historically black colleges and universities or members of the Society for Advancement of Chicanos and Native Americans in Science, George said. She noted that several AAAS programs, including the ENTRY POINT! internships for students with disabilities and the AAAS Minority Science Writers internships, work directly to prepare underrepresented scientists for the global workforce.

In 2012, a working group of the U.S. National Institutes of Health Advisory Committee to the Director suggested that more intensive career preparation would be especially valuable in building wider science participation among underrepresented groups. It recommended that all NIH grants that support postdoctoral researchers include a career-building tool called an individual development plan, or IDP.

myIDP, launched last fall at the *Science* Careers Web site, is the only online site to offer an IDP tailored for scientists. Nearly 30,000 users—roughly equivalent to one-third of the U.S. postdoc population—have registered to use myIDP. The site offers exercises and advice to scientists on how to “match up their own skills, their own interests and their own values with a variety of available career paths,” said Jim Austin, *Science* Careers’ editor.

Austin worked on the site with researchers from the University of California, San Francisco, the Medical College of Wisconsin and the Federation of American Societies for Experimental Biology who had been tracking the need for a professional development tool for a decade. The American Chemical Society and the U.S. National Science Foundation, along with the NIH, have expressed interest in using IDPs, Austin said.

“A lot of us knew of the frustration that was out there,” he said. “We knew that scientists training in academia could no longer depend on the types of jobs that they were traditionally trained for, and would need to do some serious thinking about where they were likely to end up.”

—Becky Ham and Kathleen O’Neil

EDUCATION

Retired Scientists Return to Elementary Classrooms

After earning a Ph.D. in atomic physics and spending nearly 20 years managing a U.S. Department of Energy plasma physics program, Ronald McKnight has returned to the seventh grade.

McKnight is one of 70 retired scientists, engineers, and physicians heading back to the classroom in Maryland and Virginia through the Senior Scientists and Engineers (SSE) volunteer program sponsored by AAAS.

SSE first started sending Ph.D.s like McKnight into public school classrooms in 2005, as an extension of its mission to give senior scientists opportunities to continue contributing to society after retirement.

Retired Jet Propulsion Laboratory chemist and current coordinator of the SSE volunteer program Donald Rea is now encouraging fellow retirees to establish similar programs around the country.

Rea has presented the SSE model, which is based on two earlier programs started in the 1990s, at various science and education conferences, including the International Teacher-Scientist Partnership Conference (ITSPC) in Boston this February and the American Chemical Society National Meeting in New Orleans this April.

"Many of our members are concerned

about the state of K-12 STEM education, and this is an opportunity for them to make a meaningful contribution," said Rea. "We are very eager to encourage communities elsewhere around the country to start up their own programs like ours."

According to Shirley Malcom, director of AAAS Education and Human Resources (EHR) and former co-chair of the National Science Board Commission on 21st Century Education in STEM, the most efficient way to boost STEM education is to connect teachers and students with real science and real-life scientists.

To this end, retired scientists are "an untapped source of talent and potential," according to Malcom.

In an effort to foster these relationships, EHR joined forces with the University of California, San Francisco Science & Education Partnership to host 400 teachers and scientists at the ITSPC during the 2013 AAAS Annual Meeting.

Program organizers invited Rea to share the success of SSE with teachers and scientists from all over the world. During a panel discussion, Rea stressed that volunteer training is the key to a successful program.

"Generally, what goes on in the classroom is very different from when [volunteers] went to school," he said.



Voices of experience. Donald Rea (left) and Ronald McKnight touted the benefits of volunteering in K-12 classrooms.

SSE volunteers attend a daylong training where they learn from experienced volunteers and the school district's science supervisors, who advise them on matters such as fostering discussion and inquiry rather than lecturing, and leaving discipline to the teacher.

In the classroom, volunteers can answer questions during small group activities, help to troubleshoot failed experiments, and give teachers license to step out from behind the teacher's edition of their textbooks.

The results are undeniable, Rea told the panel audience. Teachers eagerly sign up to participate year after year, volunteers report a great deal of personal satisfaction, and the students reap the rewards.

—Noelle Swan

PUBLIC ENGAGEMENT

Jumping for Jelly Beans at the White House

Lured by the promise of jelly beans, children crowded the AAAS table on the South Lawn of the White House during the 135th annual White House Easter Egg Roll on 1 April. AAAS staff explained that it takes about 30 seconds of aerobic activity to burn four calories, and led kids in half a minute of jumping in order to earn a paper cup with four of the tiny sweets. All of the AAAS activities for the White House Easter Egg Roll came from Science Gym, a program developed to encourage science and physical education teachers to work together, which was produced with support from the Golden Grant, an internal AAAS program to support new and innovative initiatives.

—Kat Zambon



A Massive Pulsar in a Compact Relativistic Binary

John Antoniadis,* Paulo C. C. Freire, Norbert Wex, Thomas M. Tauris, Ryan S. Lynch, Marten H. van Kerkwijk, Michael Kramer, Cees Bassa, Vik S. Dhillon, Thomas Driebe, Jason W. T. Hessels, Victoria M. Kaspi, Vladislav I. Kondratiev, Norbert Langer, Thomas R. Marsh, Maura A. McLaughlin, Timothy T. Pennucci, Scott M. Ransom, Ingrid H. Stairs, Joeri van Leeuwen, Joris P. W. Verbiest, David G. Whelan

Introduction: Neutron stars with masses above 1.8 solar masses ($M_{\odot}$), possess extreme gravitational fields, which may give rise to phenomena outside general relativity. Hitherto, these strong-field deviations have not been probed by experiment, because they become observable only in tight binaries containing a high-mass pulsar and where orbital decay resulting from emission of gravitational waves can be tested. Understanding the origin of such a system would also help to answer fundamental questions of close-binary evolution.

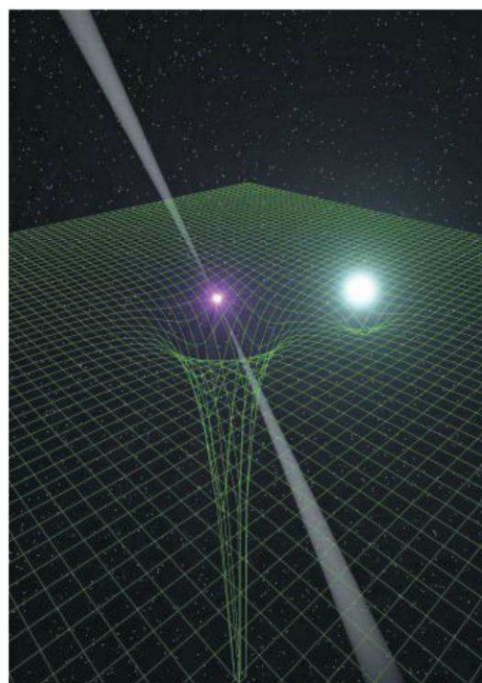
Methods: We report on radio-timing observations of the pulsar J0348+0432 and phase-resolved optical spectroscopy of its white-dwarf companion, which is in a 2.46-hour orbit. We used these to derive the component masses and orbital parameters, infer the system's motion, and constrain its age.

Results: We find that the white dwarf has a mass of $0.172 \pm 0.003 M_{\odot}$, which, combined with orbital velocity measurements, yields a pulsar mass of $2.01 \pm 0.04 M_{\odot}$. Additionally, over a span of 2 years, we observed a significant decrease in the orbital period, $\dot{P}_b^{\text{obs}} = -8.6 \pm 1.4 \mu\text{s year}^{-1}$ in our radio-timing data.

Discussion: Pulsar J0348+0432 is only the second neutron star with a precisely determined mass of $2 M_{\odot}$ and independently confirms the existence of such massive neutron stars in nature. For these masses and orbital period, general relativity predicts a significant orbital decay, which matches the observed value, $\dot{P}_b^{\text{obs}}/\dot{P}_b^{\text{GR}} = 1.05 \pm 0.18$.

The pulsar has a gravitational binding energy 60% higher than other known neutron stars in binaries where gravitational-wave damping has been detected. Because the magnitude of strong-field deviations generally depends nonlinearly on the binding energy, the measurement of orbital decay transforms the system into a gravitational laboratory for an as-yet untested gravity regime. The consistency of the observed orbital decay with general relativity therefore supports its validity, even for such extreme gravity-matter couplings, and rules out strong-field phenomena predicted by physically well-motivated alternatives. Moreover, our result supports the use of general relativity-based templates for the detection of gravitational waves from merger events with advanced ground-based detectors.

Lastly, the system provides insight into pulsar-spin evolution after mass accretion. Because of its short merging time scale of 400 megayears, the system is a direct channel for the formation of an ultracompact x-ray binary, possibly leading to a pulsar-planet system or the formation of a black hole.



Artist's impression of the PSR J0348+0432 system. The compact pulsar (with beams of radio emission) produces a strong distortion of spacetime (illustrated by the green mesh). Conversely, spacetime around its white dwarf companion (in light blue) is substantially less curved. According to relativistic theories of gravity, the binary system is subject to energy loss by gravitational waves.

READ THE FULL ARTICLE ONLINE
<http://dx.doi.org/10.1126/science.1233232>

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Fig. 2. Mass measurement of the white dwarf companion to PSR J0348+0432

Fig. 3. System masses and orbital-inclination constraints

Fig. 4. Probing strong field gravity with PSR J0348+0432

Fig. 5. Constraints on the phase offset in gravitational wave cycles in the LIGO/VIRGO bands

Fig. 6. Past and future orbital evolution of PSR J0348+0432

Fig. 7. Possible formation channels and final fate of PSR J0348+0432

Table 1. Observed and derived parameters for the PSR J0348+0432 system

SUPPLEMENTARY MATERIALS

Supplementary Text

Figs. S1 to S11

Tables S1 to S3

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ADDITIONAL RESOURCES

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A Massive Pulsar in a Compact Relativistic Binary

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Many physically motivated extensions to general relativity (GR) predict substantial deviations in the properties of spacetime surrounding massive neutron stars. We report the measurement of a 2.01 ± 0.04 solar mass ($M_\odot$) pulsar in a 2.46-hour orbit with a $0.172 \pm 0.003 M_\odot$ white dwarf. The high pulsar mass and the compact orbit make this system a sensitive laboratory of a previously untested strong-field gravity regime. Thus far, the observed orbital decay agrees with GR, supporting its validity even for the extreme conditions present in the system. The resulting constraints on deviations support the use of GR-based templates for ground-based gravitational wave detectors. Additionally, the system strengthens recent constraints on the properties of dense matter and provides insight to binary stellar astrophysics and pulsar recycling.

Neutron stars (NSs) with masses above 1.8 solar mass ($M_\odot$) manifested as radio pulsars are valuable probes of fundamental physics in extreme conditions unique in the observable universe and inaccessible to terrestrial experiments. Their high masses are directly linked to the equation-of-state (EOS) of matter at supra-nuclear densities (1, 2) and constrain the lower mass limit for production of astrophysical black holes (BHs). Furthermore, they possess extreme internal gravitational fields, which result in gravitational binding energies substantially higher than those found in more common, $1.4 M_\odot$ NSs. Modifications to general relativity (GR), often motivated by the desire for a unified model of the four

fundamental forces, can generally imprint measurable signatures in the gravitational waves (GWs) radiated by systems containing such objects, even if deviations from GR vanish in the solar system and in less-massive NSs (3–5).

However, the most massive NSs known today reside in long-period binaries or other systems unsuitable for GW radiation tests. Identifying a massive NS in a compact, relativistic binary is thus of key importance for understanding gravity-matter coupling under extreme conditions. Furthermore, the existence of a massive NS in a relativistic orbit can also be used to test current knowledge of close binary evolution.

Results

PSR J0348+0432 and Optical Observations of Its Companion

PSR J0348+0432, a pulsar spinning at 39 ms in a 2.46-hour orbit with a low-mass companion, was detected by a recent survey (6, 7) conducted with the Robert C. Byrd Green Bank Telescope (GBT). Initial timing observations of the binary yielded an accurate astrometric position, which allowed us to identify its optical counterpart in the Sloan Digital Sky Survey (SDSS) archive (8). The colors and flux of the counterpart are consistent with a low-mass white dwarf (WD) with a helium core at a distance of $d \sim 2.1$ kpc. Its relatively high apparent brightness ($g' = 20.71 \pm 0.03$ mag) allowed us to resolve its spectrum by using the Apache Point Optical Telescope. These observations revealed deep hydrogen lines, typical of low-mass WDs, confirming our preliminary identification. The radial velocities of the WD mirrored that of PSR J0348+0432, also verifying that the two stars are gravitationally bound.

In December 2011, we obtained phase-resolved spectra of the optical counterpart by using the

FORS2 spectrograph of the Very Large Telescope (VLT). For each spectrum, we measured the radial velocity, which we then folded modulo the system's orbital period. Our orbital fit to the velocities constrains the semiamplitude of their modulation to be $K_{WD} = 351 \pm 4 \text{ km s}^{-1}$ (1- σ confidence interval) (Fig. 1; see also Materials and Methods). Similarly, the orbital solution from radio-pulsar timing yields $K_{PSR} = 30.008235 \pm 0.000016 \text{ km s}^{-1}$ for the pulsar. Combined, these constraints imply a mass ratio, $q = M_{PSR}/M_{WD} = K_{WD}/K_{PSR} = 11.70 \pm 0.13$.

Modeling of the Balmer-series lines in a high signal-to-noise average spectrum formed by the coherent addition of individual spectra (Fig. 1B) shows that the WD has an effective temperature of $T_{\text{eff}} = 10,120 \pm 47_{\text{stat}} \pm 90_{\text{sys}}$ K and a surface gravity of $\log_{10} [g \text{ (cm s}^{-2}\text{)}] = 6.035 \pm 0.032_{\text{stat}} \pm 0.060_{\text{sys}}$ dex. Here, the systematic error is an overall estimate of uncertainties resulting from our fitting technique and flux calibration (8). We found no correlation of this measurement with orbital phase and no signs of rotationally induced broadening in the spectral lines (8). Furthermore, we searched for variability by using the ULTRACAM instrument (9) on the 4.2-m William-Herschel Telescope in La Palma, Spain. The light curves (fig. S3), spanning 3 hours in total, have a root-mean-square (rms) scatter of ~ 0.53 , 0.07, and 0.08 mag in u' , g' , and r' , respectively, and show no evidence for variability over the course of the observations. The phase-folded light curve shows no variability either. Additionally, our calibrated magnitudes are consistent with the SDSS catalog magnitudes, implying that the WD shone at a constant flux over this ~ 5 year time scale (8).

Mass of the White Dwarf

The surface gravity of the WD scales with its mass and the inverse square of its radius ($g \equiv GM_{WD}/R_{WD}^2$, where G is Newton's gravitational constant). Thus, the observational constraints combined with a theoretical finite-temperature mass-radius relation for low-mass WDs yield a unique solution for the mass of the companion (10). Numerous such models exist in the literature, the most detailed of which are in good agreement for very-low-mass WDs (<0.17 to $0.18 M_\odot$) but differ substantially for higher masses [e.g., (11–13)]. The main reason for this is the difference in the predicted size of the hydrogen envelope, which determines whether the main energy source of the star is residual hydrogen burning (for “thick” envelopes) or the latent heat of the core (for “thin” envelopes).

In the most widely accepted scenario, WDs lose their thick hydrogen envelope only if their mass exceeds a threshold. The exact location of the latter is still uncertain but estimated to be around 0.17 to $0.22 M_\odot$ [e.g., (11–13)]. Two other pulsars with WD companions, studied in the literature, strongly suggest that this transition threshold is indeed most likely close to $0.2 M_\odot$ (10, 14). In particular, PSR J1909–3744 has a large characteristic age of several gigayears (Gy) and a WD

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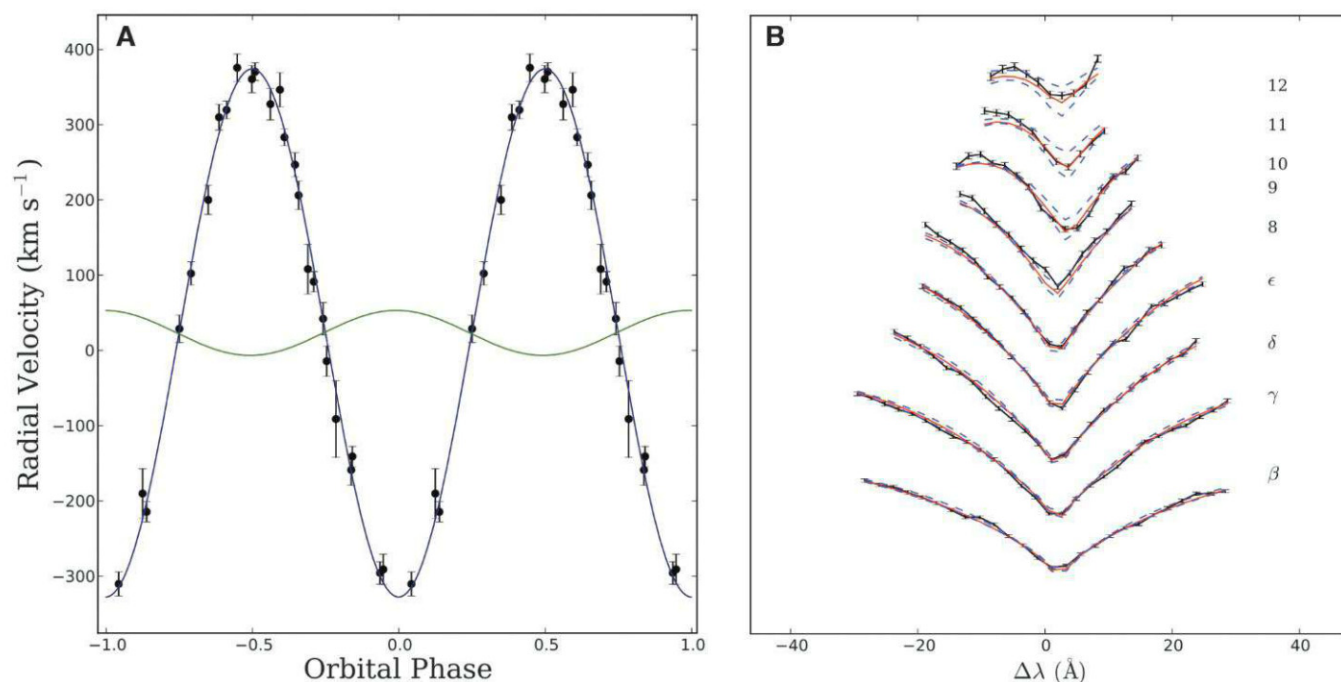


Fig. 1. Radial velocities and spectrum of the white dwarf companion to PSR J0348+0432. (A) Radial velocities of the WD companion to PSR J0348+0432 plotted against the orbital phase (shown twice for clarity). Overplotted is the best-fit orbit of the WD (blue line) and the mirror orbit of the pulsar (green). Error bars indicate 1- σ confidence intervals. (B) Details of the fit to the Balmer lines (H β to H12) in the average spectrum of the WD companion

to PSR J0348+0432 created by the coherent addition of 26 individual spectra shifted to zero velocity. Lines from H β (bottom) to H12 are shown. The red solid lines are the best-fit atmospheric model (see text). Two models, one with $T_{\text{eff}} = 9900$ K and $\log_{10} g = 5.70$ and one with $T_{\text{eff}} = 10,200$ K and $\log_{10} g = 6.30$, each ~ 3 - σ off from the best-fit central value (including systematics), are shown for comparison (dashed blue lines).

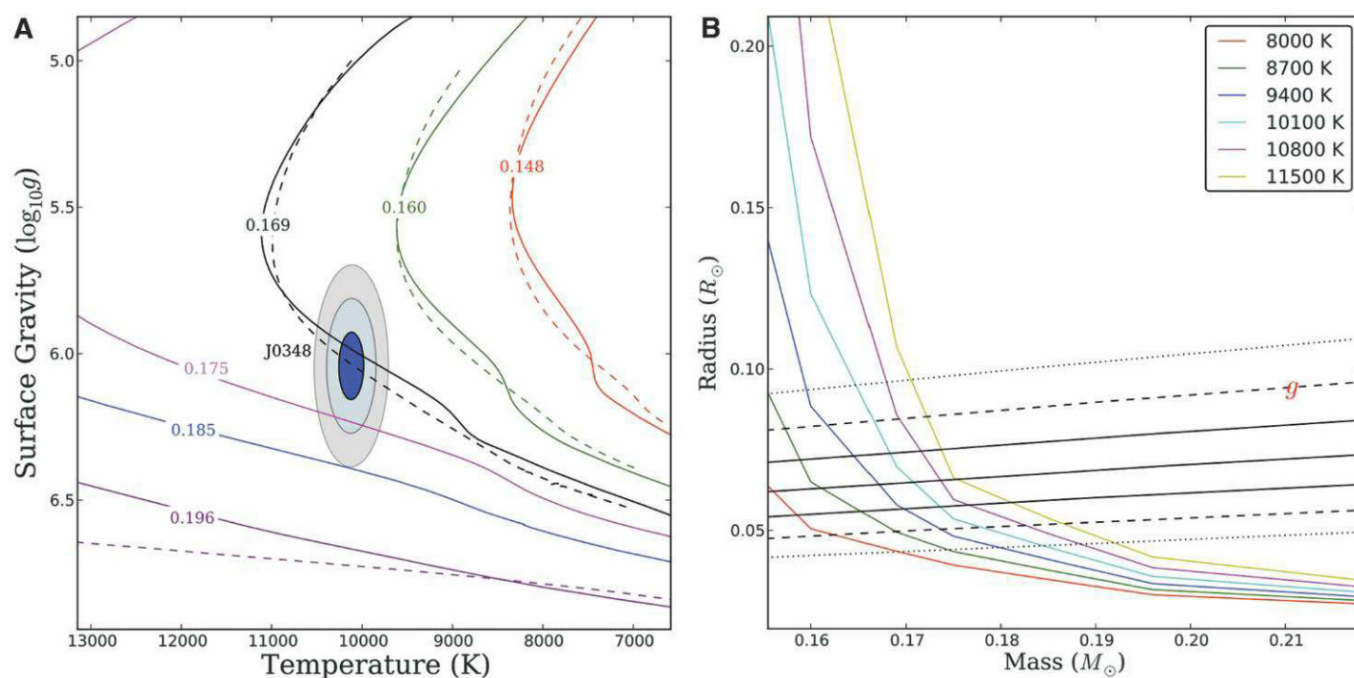


Fig. 2. Mass measurement of the white dwarf companion to PSR J0348+0432. (A) Constraints on T_{eff} and g for the WD companion to PSR J0348+0432 compared with theoretical WD models. The shaded areas depict the $\chi^2 - \chi^2_{\text{min}} = 2.3, 6.2$, and 11.8 intervals (equivalent to 1-, 2-, and 3- σ) of our fit to the average spectrum. Dashed lines show the detailed theoretical

cooling models of (11). Continuous lines depict tracks with thick envelopes for masses up to $\sim 0.2 M_{\odot}$ that yield the most conservative constraints for the mass of the WD. (B) Finite-temperature mass-radius relations for our models together with the constraints imposed from modeling of the spectrum. Low mass-high temperature points are an extrapolation from lower temperatures.

companion with a well-determined mass of $0.20 M_{\odot}$ (15) that appears to be hot (10), suggesting that its envelope is thick. For this reason, we base the WD mass estimate on cooling tracks with thick hydrogen atmospheres for masses up to $0.2 M_{\odot}$, which we constructed by using the MESA stellar evolution code (8, 16). Initial models were built for masses identical to the ones in (11), for which previous comparisons have yielded good agreement with observations (14), with the addition of tracks with 0.175 and 0.185 $M_{\odot}$ for finer coverage (Fig. 2). For masses up to 0.169 $M_{\odot}$, our models show excellent agreement with (11); however, our 0.196 $M_{\odot}$ model is quite different, because it has a thick envelope instead of a thin one. Being closer to the constraints for the WD companion to PSR J0348+0432, it yields a more conservative mass constraint, $M_{\text{WD}} = 0.165$ to 0.185 at 99.73% confidence (Fig. 3 and Table 1), which we adopt. The corresponding radius is $R_{\text{WD}} = 0.046$ to 0.092 $R_{\odot}$ at 99.73% confidence. Our models yield a cooling age of $\tau_{\text{cool}} \sim 2$ Gy.

Pulsar Mass

The derived WD mass and the observed mass ratio q imply a NS mass in the range from 1.97 to 2.05 $M_{\odot}$ at 68.27% or 1.90 to 2.18 $M_{\odot}$ at 99.73% confidence. Hence, PSR J0348+0432 is only the second NS with a precisely determined mass around 2 $M_{\odot}$, after PSR J1614–2230 (2). It has a 3- σ lower mass limit 0.05 $M_{\odot}$ higher than the latter and therefore provides a verification, using a different method, of the constraints on the EOS of superdense matter present in NS interiors (2, 17). For these masses and the known orbital period, GR predicts that the orbital period should decrease

at the rate of $\dot{P}_b^{\text{GR}} = (-2.58^{+0.07}_{-0.11}) \times 10^{-13} \text{ s s}^{-1}$ (68.27% confidence) because of energy loss through GW emission.

Radio Observations

Since April 2011, we have been observing PSR J0348+0432 with the 1.4-GHz receiver of the 305-m radio telescope at the Arecibo Observatory by using its four wide-band pulsar processors (18). In order to verify the Arecibo data, we have been independently timing PSR J0348+0432 at 1.4 GHz by using the 100-m radio telescope in Effelsberg, Germany. The two timing data sets produce consistent rotational models, providing added confidence in both. Combining the Arecibo and Effelsberg data with the initial GBT observations (7), we derived the timing solution presented in Table 1. To match the arrival times, the solution requires a significant measurement of orbital decay, $\dot{P}_b = -2.73 \times 10^{-13} \pm 0.45 \times 10^{-13} \text{ s s}^{-1}$ (68.27% confidence).

The total proper motion and distance estimate (Table 1) allowed us to calculate the kinematic corrections to $\dot{P}_b$ from its motion in the Galaxy, plus any contribution from possible variations of G : $\delta\dot{P}_b = 0.016 \times 10^{-13} \pm 0.003 \times 10^{-13} \text{ s s}^{-1}$. This is negligible compared to the measurement uncertainty. Similarly, the small rate of rotational energy loss of the pulsar (Table 1) excludes any substantial contamination resulting from mass loss from the system; furthermore, we can exclude substantial contributions to $\dot{P}_b$ from tidal effects [see (8) for details]. Therefore, the observed $\dot{P}_b$ is caused by GW emission, and its magnitude is entirely consistent with the one predicted by GR: $\dot{P}_b/\dot{P}_b^{\text{GR}} = 1.05 \pm 0.18$ (Fig. 3).

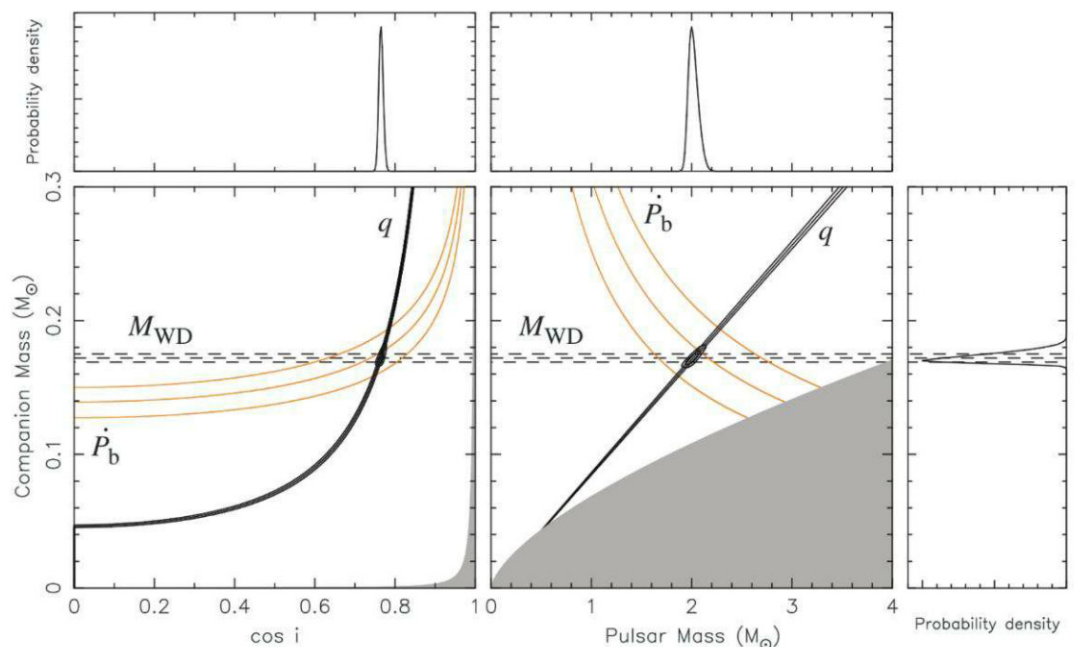
If we assume that GR is the correct theory of gravity, we can then derive the component masses from the intersection of the regions allowed by q and $\dot{P}_b$ (Fig. 3): $M_{\text{WD}} = 0.177^{+0.017}_{-0.018} M_{\odot}$ and $M_{\text{PSR}} = 2.07^{+0.20}_{-0.21} M_{\odot}$ (68.27% confidence). These values are not too constraining yet. However, the uncertainty of the measurement of $\dot{P}_b$ decreases with $T_{\text{baseline}}^{-5/2}$ (where T_{baseline} is the timing baseline); therefore, this method will yield very precise mass measurements within a couple of years.

Discussion

PSR J0348+0432 as a Testbed for Gravity

There are strong arguments for GR not to be valid beyond a (yet unknown) critical point, like its incompatibility with quantum theory and its prediction of the formation of spacetime singularities. Therefore, it remains an open question whether GR is the final description of macroscopic gravity. This strongly motivates testing gravity regimes that have not been tested before, in particular regimes where gravity is strong and highly nonlinear. Presently, binary pulsars provide the best high-precision experiments to probe strong-field deviations from GR and the best tests of the radiative properties of gravity (19–23). The orbital period of PSR J0348+0432 is only 15 s longer than that of the double pulsar system PSR J0737–3039, but it has ~two times more fractional gravitational binding energy than each of the double-pulsar NSs. This places it far outside the presently tested binding energy range (Fig. 4A) (8). Because the magnitude of strong-field effects generally depends nonlinearly on the binding energy, the measurement of orbital decay transforms the

Fig. 3. System masses and orbital-inclination constraints. Constraints on system masses and orbital inclination from radio and optical measurements of PSR J0348+0432 and its WD companion. Each triplet of curves corresponds to the most likely value and standard deviations (68.27% confidence) of the respective parameters. Of these, two (q and M_{WD}) are independent of specific gravity theories (in black). The contours contain the 68.27 and 95.45% of the two-dimensional probability distribution. The constraints from the measured intrinsic orbital decay ($\dot{P}_b^{\text{int}}$, in orange) are calculated assuming that GR is the correct theory of gravity. All curves intersect in the same region, meaning that GR passes this radiative test (8). **(Bottom left)** $\cos i$ - M_{WD} plane. The gray region is excluded by the condition $M_{\text{PSR}} > 0$. **(Bottom right)** M_{PSR} - M_{WD} plane. The gray region is excluded by the condition $\sin i \leq 1$. The lateral graphs depict the one-dimensional probability-distribution function for the WD mass (right), pulsar mass (top right), and inclination (top left) based on the mass function, M_{WD} , and q .



system into a gravitational laboratory for a previously untested regime, qualitatively very different from what was accessible in the past.

In physically consistent and extensively studied alternatives, gravity is generally mediated by extra fields (e.g., scalar) in addition to the tensor field of GR (5). A dynamical coupling between matter and these extra fields can lead to prominent deviations from GR that only occur at the high gravitational binding energies of massive NSs. One of the prime examples is the strong-field scalarization discovered in (3). If GR is not valid, in the PSR J0348+0432 system where such an object is closely orbited by a weakly self-gravitating body, one generally expects a violation of the strong equivalence principle that in turn leads to a modification in the emission of GWs. Although in GR the lowest source multipole that generates gravitational radiation is the quadrupole, alternative gravity theories generally predict the presence of monopole and dipole radiation on top of a modification of the other multipoles (5). For a binary system, the leading change in the orbital period is then given by the dipole contribution, which for a (nearly) circular orbit reads (8)

$$\dot{P}_b^{\text{dipolar}} \simeq -\frac{4\pi^2 G}{c^3 P_b} \frac{M_{\text{PSR}} M_{\text{WD}}}{M_{\text{PSR}} + M_{\text{WD}}} (\alpha_{\text{PSR}} - \alpha_{\text{WD}})^2 \quad (1)$$

where α_{PSR} is the effective coupling strength between the NS and the ambient fields responsible for the dipole moment (e.g., scalar fields in scalar-tensor gravity) and α_{WD} is the same parameter for the WD companion. The WD companion to PSR J0348+0432 has a fractional gravitational binding energy ($E_{\text{grav}}/M_{\text{WD}}c^2$) of just $\sim 1.2 \times 10^{-5}$ and is therefore a weakly self-gravitating object. Consequently, α_{WD} is practically identical to the linear field-matter coupling α_0 , which is well constrained ($|\alpha_0| < 0.004$) in solar system experiments (20, 24).

For α_{PSR} , the situation is very different. Even if α_0 is vanishingly small, α_{PSR} can have values close to unity, because of a nonlinear behavior of gravity in the interaction between matter and the gravitational fields in the strong-gravity regime inside NSs (3, 4). A significant α_{PSR} for NSs up to $1.47 M_\odot$ has been excluded by various binary pulsar experiments (8, 23). The consistency of the observed GW damping ($\dot{P}_b$) with the GR predictions for PSR J0348+0432 (Table 1) implies $|\alpha_{\text{PSR}} - \alpha_0| < 0.005$ (95% confidence) and consequently excludes significant strong-field deviations, even for massive NSs of $\sim 2 M_\odot$.

To demonstrate in some detail the implications of our results for possible strong-field deviations of gravity from Einstein's theory, we confront our limits on dipolar radiation with a specific class of scalar-tensor theories, in which gravity is mediated by a symmetric second-rank tensor field, $g_{\mu\nu}^*$, and by a long-range (massless) scalar field, ϕ . Scalar-tensor theories are well

Table 1. Observed and derived parameters for the PSR J0348+0432 system. Timing parameters for the PSR J0348+0432 system, indicated with their 1- σ uncertainties as derived by tempo2 where appropriate (numbers in parentheses refer to errors on the last digits). The timing parameters are calculated for the reference epoch modified Julian date (MJD) 56000 and are derived from TOAs in the range MJD 54872 to 56208.

<i>Optical parameters</i>	
Effective temperature, T_{eff} (K)	$10120 \pm 47_{\text{stat}} \pm 90_{\text{sys}}$
Surface gravity, $\log_{10}[g(\text{cm s}^{-1})]$	$6.035 \pm 0.032_{\text{stat}} \pm 0.060_{\text{sys}}$
Semiamplitude of orbital radial velocity, K_{WD} (km s $^{-1}$)	351 ± 4
Systemic radial velocity relative to the Sun, γ (km s $^{-1}$)	-1 ± 20
<i>Timing parameters</i>	
Right ascension, α (J2000)	$03^{\text{h}} 48^{\text{m}} 43^{\text{s}}.639000(4)$
Declination, δ (J2000)	$+04^\circ 32' 11.4580(2)$
Proper motion in right ascension, μ_α (mas year $^{-1}$)	$+4.04(16)$
Proper motion in declination, μ_δ (mas year $^{-1}$)	$+3.5(6)$
Parallax, π_d (mas)	0.47^*
Spin frequency, ν (Hz)	$25.5606361937675(4)$
First derivative of ν , $\dot{\nu}$ (10^{-15} Hz s $^{-1}$)	$-0.15729(3)$
Dispersion measure, DM (cm $^{-3}$ pc)	$40.46313(11)$
First derivative of DM, DM1 (cm $^{-3}$ pc year $^{-1}$)	$-0.00069(14)$
Orbital period, P_b (day)	$0.102424062722(7)$
Time of ascending node, T_{asc} (MJD)	$56000.084771047(11)$
Projected semimajor axis of the pulsar orbit, x (lt-s)	$0.14097938(7)$
$\eta \equiv e \sin \omega$	$+1.9 \times 10^{-6} \pm 1.0 \times 10^{-6}$
$\kappa \equiv e \cos \omega$	$+1.4 \times 10^{-6} \pm 1.0 \times 10^{-6}$
First derivative of P_b , $\dot{P}_b$ (10^{-12} s s $^{-1}$)	$-0.273(45)$
<i>Derived parameters</i>	
Galactic longitude, l	$183.^\circ 3368$
Galactic latitude, b	$-36.^\circ 7736$
Distance, d (kpc)	$2.1(2)$
Total proper motion, μ (mas year $^{-1}$)	$5.3(4)$
Spin period, P (ms)	$39.1226569017806(5)$
First derivative of P , $\dot{P}$ (10^{-18} s s $^{-1}$)	$0.24073(4)$
Characteristic age, τ_c (Gy)	2.6
Transverse magnetic field at the poles, B_0 (10^9 G)	~ 2
Rate or rotational energy loss, $\dot{E}$ (10^{32} erg s $^{-1}$)	~ 1.6
Mass function, f ($M_\odot$)	$0.000286778(4)$
Mass ratio, $q \equiv M_{\text{PSR}}/M_{\text{WD}}$	$11.70(13)$
White dwarf mass, M_{WD} ($M_\odot$)	$0.172(3)$
Pulsar mass, M_{PSR} ($M_\odot$)	$2.01(4)$
"Range" parameter of Shapiro delay, r (μ s)	0.84718^*
"Shape" parameter of Shapiro delay, $s \equiv \sin i$	0.64546^*
White dwarf radius, R_{WD} ($R_\odot$)	$0.065(5)$
Orbital separation, a (10^9 m)	0.832
Orbital separation, a ($R_\odot$)	1.20
Orbital inclination, i	$40.^\circ 2(6)$
$\dot{P}_b$ predicted by GR, $\dot{P}_b^{\text{GR}}$ (10^{-12} s s $^{-1}$)	$-0.258_{-0.011}^{+0.008}$
$\dot{P}_b/\dot{P}_b^{\text{GR}}$	1.05 ± 0.18
Time until coalescence, τ_m (My)	~ 400

*For these timing parameters, we adopted the optically derived parameters (see text for details).

motivated and consistent theories of gravity, extensively studied in the literature [e.g., (25, 26)]. For this reason, they are the most natural framework for us to illustrate the gravitational phenomena that can be probed with PSR J0348+0432.

Concerning the EOS of NS matter, in our calculations we use the rather stiff EOS "20" of (27) that supports (in GR) NSs of up to $2.6 M_\odot$. We make this choice for two reasons: (i) A stiffer EOS generally leads to more conservative limits when constraining alternative gravity theories, and (ii)

it is able to support even more massive NSs than PSR J0348+0432, which are likely to exist (28–30). Furthermore, in most of our conclusions a specific EOS is used only for illustrative purposes, and the obtained generic results are EOS independent.

Figure 4B illustrates how PSR J0348+0432 probes a nonlinear regime of gravity that has not been tested before. A change in EOS and gravity theory would lead to a modified functional shape for α_{PSR} . However, this would not change the general picture: Even in the strong gravitational

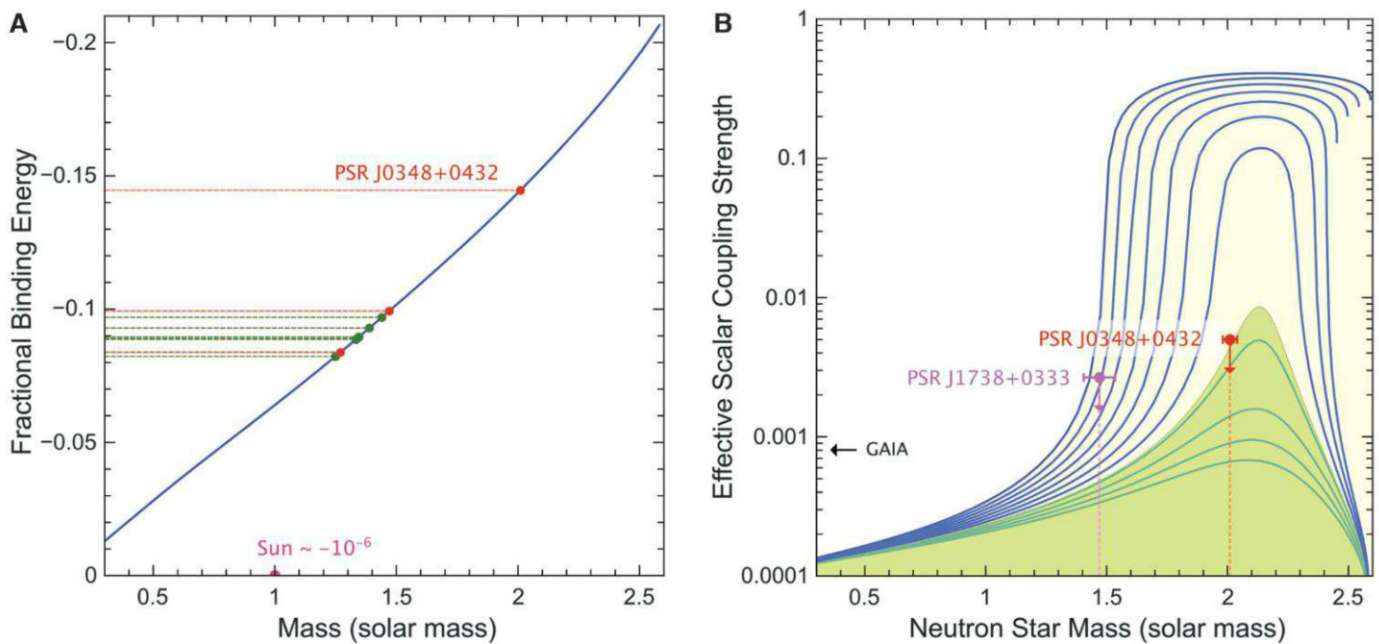


Fig. 4. Probing strong field gravity with PSR J0348+0432. (A) Fractional gravitational binding energy as a function of the inertial mass of a NS in GR (blue curve). The dots indicate the NSs of relativistic NS-NS (in green) and NS-WD (in red) binary-pulsar systems currently used for precision gravity tests (8). (B) Effective scalar coupling as a function of the NS mass, in the “quadratic” scalar-tensor theory of (4). For the linear coupling of matter to the

scalar field, we have chosen $\alpha_0 = 10^{-4}$, a value well below the sensitivity of any near-future solar system experiment [e.g., GAIA (62)]. The solid curves correspond to stable NS configurations for different values of the quadratic coupling β_0 : -5 to -4 (top to bottom) in steps of 0.1 . The yellow area indicates the parameter space allowed by the best current limit on $|\alpha_{\text{PSR}} - \alpha_0|$ (23), whereas only the green area is in agreement with the limit presented here.

Fig. 5. Constraints on the phase offset in gravitational wave cycles in the LIGO/VIRGO bands. Maximum offset in GW cycles in the LIGO/VIRGO band (20 Hz to a few kHz) between the GR template and the true phase evolution of the in-spiral in the presence of dipolar radiation as a function of the effective coupling of the massive NS for two different system configurations: a $2 M_\odot$ NS with a $1.25 M_\odot$ NS (NS-NS) and a merger of a $2 M_\odot$ NS with a $10 M_\odot$ BH (NS-BH).

In the NS-NS case, the green line is for $\alpha_B = \alpha_0$, and the gray dotted line represents the most conservative, rather unphysical, assumption $\alpha_0 = 0.004$ and $\alpha_B = 0$ (8). In the NS-BH case, α_B is set to zero (from the assumption that no-hair theorems hold). The blue line is for $\alpha_0 = 0.004$ (solar system limit for scalar-tensor theories), and the purple line represents $\alpha_0 = 0$. The gray area to the right of the red line is excluded by PSR J0348+0432. In this plot, there is no assumption concerning the EOS.

field of a $2 M_\odot$ NS, gravity seems to be well described by GR, and there is little space for any deviations, at least in the form of long-range fields, which influence the binary dynamics.

Short-range interactions, like massive Brans-Dicke gravity (31) with a sufficiently large scalar mass (heavier than $\sim 10^{-19}$ eV/ c^2), cannot be excluded by PSR J0348+0432.

Constraints on the Phase Evolution of Neutron Star Mergers

One of the most promising sources of GWs that might be detected by ground-based laser interferometers like the LIGO [Laser Interferometer Gravitational Wave Observatory (32)] and the VIRGO (33) projects (34) are in-spiraling compact binaries, consisting of NSs and BHs, whose orbits are decaying toward a final coalescence because of GW damping. As the signal sweeps in frequency through the detectors’ typical sensitive bandwidth between about 20 Hz and a few kHz, the GW signal will be deeply buried in the broadband noise of the detectors (34). To detect it, one will have to apply a matched filtering technique, that is, correlate the output of the detector with a template wave form. Consequently, it is crucial to know the binary’s orbital phase with high accuracy for searching and analyzing the signals from in-spiraling compact binaries. Typically, one aims to lose less than one GW cycle in a signal with $\sim 10^4$ cycles. For this reason, within GR such calculations have been conducted with great effort by various groups up to the 3.5 post-Newtonian order, that is, all (nonvanishing) terms up to order $(v/c)^7$ (where v is relative orbital velocity of the binary’s components), providing sufficient accuracy for a detection (35–37).

If the gravitational interaction between two compact masses is different from GR, the phase evolution over the last few thousand cycles, which fall into the bandwidth of the detectors, might be too different from the (GR) template to extract

the signal from the noise. In scalar-tensor gravity, for instance, the evolution of the phase is driven by radiation reaction, which is modified because the system loses energy to scalar GWs (38, 39). Depending on the difference between the effective scalar couplings of the two bodies, α_A and α_B , the 1.5 post-Newtonian dipolar contribution to the phase evolution could drive the GW signal many cycles away from the GR template. For this reason, it is desirable that potential deviations from GR in the interaction of two compact objects can be tested and constrained before the start of the advanced GW detectors. For “canonical” $1.4 M_\odot$ NSs and long-range gravitational fields, this has already been achieved to a high degree in binary pulsar experiments, for example, (39). So far, the best constraints on dipolar gravitational wave damping in compact binaries come from the observations of the millisecond pulsar PSR J1738+0333 (23). However, as discussed in detail above, these timing experiments are insensitive to strong-field deviations that might only become relevant in the strong gravitational fields associated with high-mass NSs. Consequently, the dynamics of a merger of a $2 M_\odot$ NS with a “canonical” NS or a BH might have a significant contribution from dipolar GWs. With our constraints on dipolar radiation damping from the timing observations of PSR J0348+0432, given above, we can already exclude a deviation of more than ~ 0.5 cycles from the GR template during the observable in-spiral caused by additional long-range gravitational fields for the whole range of NS masses observed in nature [see Fig. 5 and (8) for the details of the calculation]. This compares to the precision of GR templates based on the 3.5 post-Newtonian approximation (35, 37). Furthermore, in an extension of the arguments in (38, 39) to massive NSs, our result implies that binary pulsar experiments are already more sensitive for testing such deviations than the upcoming advanced GW detectors.

Lastly, as mentioned before, our results on PSR J0348+0432 cannot exclude dipolar radiation from short-range fields. Hence, if the range of the additional field in the gravitational interaction happens to lie between the wavelength of the GWs of PSR J0348+0432 and the wavelength of the merger signal ($\sim 10^9$ cm; $\sim 10^{-13}$ eV/c²), then the considerations concerning the applicability of the GR template given here do not apply. On the other hand, in such a case the combination of binary pulsar and LIGO/VIRGO experiments can be used to constrain the mass of this extra field.

Formation and Past and Future Evolution of the System

The measured spin period, P , and spin-period derivative $\dot{P}$ of PSR J0348+0432, combined with the masses and orbital period of the system (Table 1), form a peculiar set of parameters that gives insight to binary stellar evolution. The short 2.46-hour orbital period is best understood from evolution via a common envelope where the NS is captured in the envelope of the WD progenitor, leading to efficient removal of orbital angular

momentum on a short time scale of $\sim 10^3$ years (40). This implies that the NS was born with an initial mass close to its current mass of $2.01 M_\odot$, because very little accretion was possible. Whereas the slow spin period of ~ 39 ms and the unusually strong magnetic field (8) of a few 10^9 G (Table 1) provide further support for this scenario, the low WD mass contradicts the standard common-envelope hypothesis by requiring a progenitor star mass smaller than $2.2 M_\odot$, because more massive stars would leave behind more massive cores (8, 41). For such low donor star masses, however, the mass ratio of the binary components is close to unity, leading to dynamically stable mass transfer without forming a common envelope (42, 43). One potential solution to this mass discrepancy for common-envelope evolution is to assume that the original mass of the WD was $\geq 0.4 M_\odot$ and that it was subsequently evaporated by the pulsar wind (44) when PSR J0348+0432 was young and energetic, right after its recycling phase (8). Such an evolution could also help explain the formation of another puzzling system, PSR J1744–3922 (45). However, we find that this scenario is quite unlikely given that the observed spectrum of the WD in PSR J0348+0432 only displays hydrogen lines, which is not expected if the WD was indeed a stripped remnant of a much more massive helium or carbon-oxygen WD. Furthermore, it is unclear why this evaporation process should have come to a complete stop when the WD reached its current mass of $0.17 M_\odot$. A speculative hypothesis to circumvent the above-mentioned problems would be a common-envelope evolution with hypercritical accretion, where $\sim 0.6 M_\odot$ of material was efficiently transferred to a $1.4 M_\odot$ NS (8, 46).

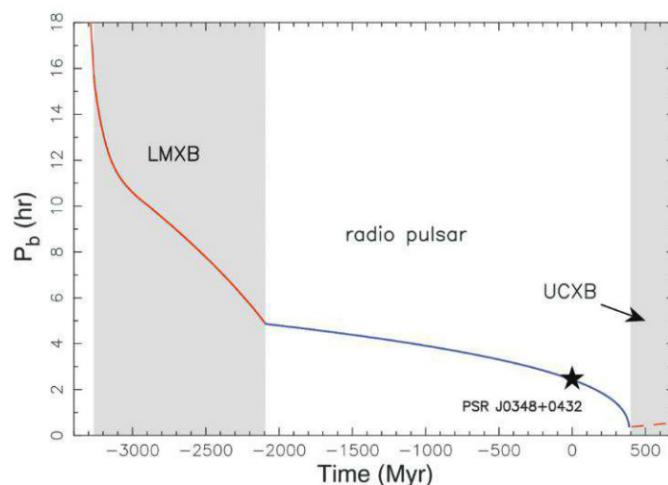
An alternative, and more promising, formation scenario is evolution via a close-orbit low-mass x-ray binary (LMXB) with a 1.0 to $1.6 M_\odot$ donor star that suffered from loss of orbital angular momentum because of magnetic braking (43, 47, 48). This requires a finely tuned truncation of the mass-transfer process, which is not

yet understood in detail but which is also required for other known recycled pulsars (8) with short orbital periods of $P_b \leq 8$ hours and low-mass helium WD companions with $M_{WD} \approx 0.14$ to $0.18 M_\odot$. The interplay between magnetic braking, angular momentum loss from stellar winds (possibly caused by irradiation), and mass ejected from the vicinity of the NS is poorly understood, and current stellar evolution models have difficulties reproducing these binary pulsar systems. One issue is that the converging LMXBs most often do not detach but keep evolving with continuous mass transfer to more and more compact systems with $P_b \leq 1$ hour and ultralight donor masses smaller than $0.08 M_\odot$.

By using the Langer stellar evolution code (8), we have attempted to model the formation of the PSR J0348+0432 system via LMXB evolution (Fig. 6). To achieve this, we forced the donor star to detach its Roche lobe at $P_b \sim 5$ hours, such that the system subsequently shrinks in size to its present value of $P_b \approx 2.46$ hours because of GW radiation within 2 Gy, the estimated cooling age of the WD. An illustration of the past and future evolution of PSR J0348+0432 from the two different formation channels is shown in Fig. 7.

An abnormality of PSR J0348+0432 in view of the LMXB model is its slow spin period of $P \sim 39$ ms and, in particular, the high value for the spin period derivative, $\dot{P} = 2.41 \times 10^{-19} \text{ s s}^{-1}$. These values correspond to an inferred surface magnetic flux density of $B \sim 2 \times 10^9$ G, which is high compared with most other recycled pulsars (49). However, a high B value naturally explains the slow spin period of PSR J0348+0432 from a combination of spin-down during the Roche-lobe decoupling phase (50) and subsequent magnetic dipole radiation from this high-magnetic-field pulsar (8, 49). Another intriguing question concerning this evolutionary channel is the spread in NS masses. In the five currently known NS-WD systems with $P_b \leq 8$ hours, the NS masses span a large range of values, ranging from ~ 1.4 up to $2.0 M_\odot$. The lower masses imply that the mass

Fig. 6. Past and future orbital evolution of PSR J0348+0432. Formation of PSR J0348+0432 from our converging LMXB model calculation. The plot shows orbital period as a function of time (calibrated to present day). The progenitor detached from its Roche lobe about 2 Gy ago (according to the estimated cooling age of the WD) when $P_b \approx 5$ hours, and since then GW damping reduced the orbital period to its present value of 2.46 hours (marked with a star). UCXB, ultracompact x-ray binary.



transfer during the LMXB phase is extremely inefficient: Only about 30% of the material leaving the donor is accreted by the NS (14, 15). If this is indeed the case and if one assumes that the physical processes that lead to the formation of these systems are similar, it is likely that PSR J0348+0432 was born with an initial mass of $1.7 \pm 0.1 M_{\odot}$, providing further support for a non-negligible fraction of NSs born massive (41).

Emission of GWs will continue to shrink the orbit of PSR J0348+0432, and in 400 My (when $P_b \approx 23$ min) the WD will fill its Roche lobe and possibly leave behind a planet orbiting the pulsar (51, 52). Alternatively, if PSR J0348+0432 is near the upper-mass limit for NSs, then a BH might form via accretion-induced collapse of the massive NS in a cataclysmic, γ -ray burst-like event (53).

Materials and Methods

Radial Velocities and Atmospheric Parameters

A detailed log of the VLT observations can be found in (8) (fig. S1 and table S1). We extracted the spectra following closely the method used in (14) and compared them with template spectra to measure the radial velocities. Our best fits for the WD had reduced χ^2 minimum values of $\chi^2_{\text{red, min}} = 1.0$ to 1.5 (8). Uncertainties were taken to be the

difference in velocity over which χ^2 increases by $\chi^2_{\text{red, min}}$ to account for the fact that $\chi^2_{\text{red, min}}$ is not equal to unity (14). After transforming the measurements to the reference frame of the solar system barycenter (SSB), we folded them by using the radio-timing ephemeris described below. We then fitted for the semiamplitude of the radial velocity modulation, K_{WD} , and the systemic radial velocity with respect to the SSB, γ , assuming a circular orbit and keeping the time of passage through the ascending node, T_{asc} , fixed to the best-fit value of the radio-timing ephemeris. Our solution yields $K_{\text{WD}} = 351 \pm 4 \text{ km s}^{-1}$ and $\gamma = -1 \pm 20 \text{ km s}^{-1}$ (8).

Details of the Balmer lines in the average spectrum of PSR J0348+0432, created by the coherent addition of the individual spectra shifted to zero velocity, are shown in Fig. 1B. We modeled the spectrum by using a grid of detailed hydrogen atmospheres (54). These models incorporate the improved treatment of pressure broadening of the absorption lines presented in (55). As mentioned above, our fit yields $T_{\text{eff}} = 10,120 \pm 35_{\text{stat}} \pm 90_{\text{sys}} \text{ K}$ for the effective temperature and $\log_{10} g = 6.042 \pm 0.032_{\text{stat}} \pm 0.060_{\text{sys}}$ for the surface gravity (8). The χ^2 map shown in Fig. 2A is inflated to take into account systematic uncertainties. The average spectrum was also searched

for rotational broadening. By using the analytic profile of (56) to convolve the model atmospheres, we scanned the grid of velocities $0 \leq v_r \sin i \leq 2000 \text{ km s}^{-1}$ with a step size of 100 km s^{-1} . The result is consistent with no rotation and our 1- σ upper limit is $v_r \sin i \leq 430 \text{ km s}^{-1}$.

Modeling of the White Dwarf Mass

Low-mass WDs are thought to form naturally within the age of the universe via mass transfer in a binary, either through Roche-lobe overflow or common-envelope evolution. In both cases, the WD forms when the envelope mass drops below a critical limit, which depends primarily on the mass of the stellar core, forcing the star to contract and detach from its Roche lobe. After the contraction, the mass of the relic envelope is fixed for a given core mass, but further reduction of its size may occur shortly before the star enters the final cooling branch because of hydrogen-shell flashes that force the star to reexpand to giant dimensions. Additional mass removal via Roche-lobe overflow, as well as rapid hydrogen-shell burning through the CNO cycle, may then lead to a decrease of the envelope size and affect the cooling history and atmospheric parameters. To investigate the consequence of a reduced envelope size for the WD companion to PSR J0348+0432, we constructed WD models in which we treat the envelope mass as a free parameter (8). For the WD companion to PSR J0348+0432, an envelope mass below the critical limit for hydrogen fusion is not likely for two main reasons.

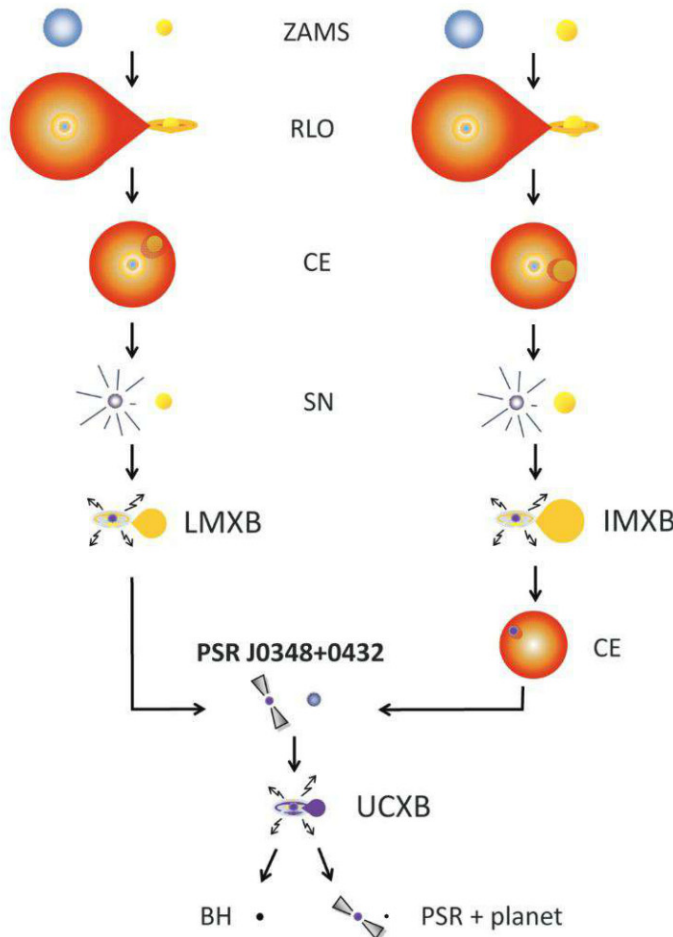
First, for a pure helium composition, the observed surface gravity translates to a WD mass of $\sim 0.15 M_{\odot}$ and a cooling age of ~ 20 My, which is anomalously small. Such a small age would also imply a large increase in the birth and in-spiral rate of similar relativistic NS-WD systems (57). Furthermore, postcontraction flash episodes on the WD are not sufficient to remove the entire envelope. Therefore, creation of a pure helium WD requires large mass loss rates before the progenitor contracts, which is unlikely. For small progenitor masses ($\leq 1.5 M_{\odot}$), large mass loss prevents contraction, and the star evolves to a semi-degenerate companion on a nuclear time scale that exceeds the age of the universe. For more massive progenitors ($> 1.5 M_{\odot}$), the core grows beyond $\sim 0.17 M_{\odot}$ in a short time scale and ultimately leaves a too-massive WD.

Second, even for envelope hydrogen fractions as low as $X_{\text{avg}} = 10^{-6}$, the observed temperature and surface gravity cannot be explained simultaneously: The low surface gravity would again require a small mass of $\sim 0.15 M_{\odot}$. However, in this case the surface hydrogen acts like an insulator, preventing the heat of the core from reaching the stellar surface. As a result, temperatures as high as 10,000 K can only be reached for masses above $\sim 0.162 M_{\odot}$.

Past a critical envelope mass, the pressure at the bottom of the envelope becomes high enough to initiate hydrogen-shell burning. The latter then becomes the dominant energy source, and the

Fig. 7. Possible formation channels and final fate of PSR J0348+0432.

An illustration of the formation and evolution of PSR J0348+0432. The zero-age main sequence (ZAMS) mass of the NS progenitor is likely to be 20 to 25 $M_{\odot}$, whereas the WD progenitor had a mass of 1.0 to 1.6 $M_{\odot}$ (LMXB) or 2.2 to 5 $M_{\odot}$ (common envelope, CE), depending on its formation channel. In ~ 400 My (when $P_b \sim 23$ min), the WD will fill its Roche lobe, and the system becomes an UCXB, leading to the formation of a BH or a pulsar with a planet. RLO, Roche-lobe overflow; SN, supernova; IMXB, intermediate-mass x-ray binary.



evolutionary time scale increases; the radius of the star grows by $\sim 50\%$ (depending on the mass), expanding further for larger envelopes. The dependence of the surface gravity on the radius implies that the observed value translates to a higher mass as the envelope mass increases. Therefore, the most conservative lower limit for the WD mass (and thus for PSR J0348+0432, given the fixed mass ratio) is obtained if one considers models with the absolute minimum envelope mass required for hydrogen burning. In this scenario, the mass of the WD is in the range from 0.162 to 0.181 $M_{\odot}$ at 99.73% confidence (8). Despite this constraint being marginally consistent with our observations (8), it is not likely correct because of the high degree of fine-tuning.

For these reasons, we have adopted the assumption that the WD companion to PSR J0348+0432 has a thick envelope, as generally expected for WDs with such low surface gravity and high temperature.

Radio Timing Analysis

The Arecibo observing setup (8) and data reduction are similar to the well-tested ones described in (23). Special care was taken with saving raw search data, which allows for iterative improvement of the ephemeris and eliminates orbital phase-dependent smearing of the pulse profiles, which might contaminate the measurement of $\dot{P}_b$ (58). From this analysis, we derived 7773 independent measurements of pulse times of arrival (TOAs) with a rms uncertainty smaller than 10 μ s. Similarly the Effelsberg observations yield a total of 179 TOAs with uncertainties smaller than 20 μ s.

We used the tempo2 timing package (59) and 8121 available TOAs from GBT (7), Arecibo, and Effelsberg to derive the timing solution presented in Table 1. The motion of the radiotelescopes relative to the barycenter of the Solar System was computed by using the DE/LE 421 solar system ephemeris (60) published by the Jet Propulsion Laboratory. The orbit of PSR J0348+0432 has a very low eccentricity; therefore, we use the ELL1 orbital model (61) to describe the motion of the pulsar.

For the best fit, the reduced χ^2 of the timing residuals (TOA minus model prediction) is 1.66, a result similar to what was obtained in timing observations of other millisecond pulsars. The overall weighted residual rms is 4.6 μ s. There are no unmodeled systematic trends in the residuals, either as a function of orbital phase or as a function of time. Therefore $\chi^2 > 1$ is most likely produced by underestimated TOA uncertainties. We increased our estimated TOA uncertainties for each telescope and receiver to produce a reduced χ^2 of unity on short time scales; for our dominant data set (Arecibo), the errors were multiplied by a factor of 1.3.

This produces more conservative estimates of the uncertainties of the timing parameters; these have been verified by using the Monte Carlo statistical method described in (23): When all pa-

rameters are fitted, the Monte Carlo uncertainty ranges are very similar to those estimated by tempo2. As an example, tempo2 estimates $\dot{P}_b = -2.73 \times 10^{-13} \pm 0.45 \times 10^{-13} \text{ s s}^{-1}$ (68.27% confidence), and the Monte Carlo method yields $\dot{P}_b = -2.72 \times 10^{-13} \pm 0.45 \times 10^{-13} \text{ s s}^{-1}$ (68.27% confidence), in excellent agreement. The observed orbital decay appears to be stable; no higher derivatives of the orbital period are detected (8).

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Supplementary Materials

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Supplementary Text

Figs. S1 to S11

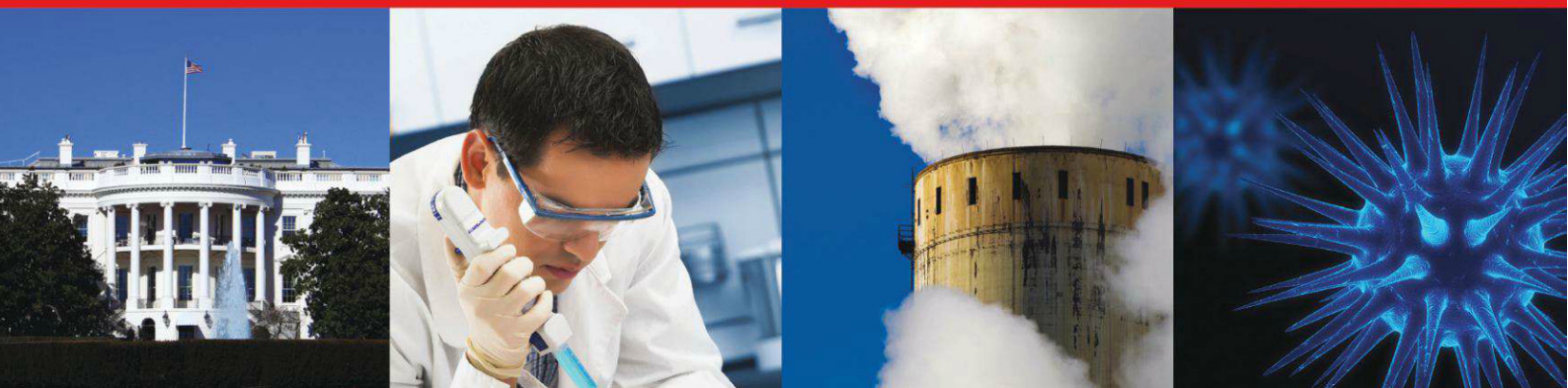
Tables S1 to S3

References (63–112)

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Neurotransmitter Switching in the Adult Brain Regulates Behavior

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Neurotransmitters have been thought to be fixed throughout life, but whether sensory stimuli alter behaviorally relevant transmitter expression in the mature brain is unknown. We found that populations of interneurons in the adult rat hypothalamus switched between dopamine and somatostatin expression in response to exposure to short- and long-day photoperiods. Changes in postsynaptic dopamine receptor expression matched changes in presynaptic dopamine, whereas somatostatin receptor expression remained constant. Pharmacological blockade or ablation of these dopaminergic neurons led to anxious and depressed behavior, phenocopying performance after exposure to the long-day photoperiod. Induction of newly dopaminergic neurons through exposure to the short-day photoperiod rescued the behavioral consequences of lesions. Natural stimulation of other sensory modalities may cause changes in transmitter expression that regulate different behaviors.

Signal transmission in neuronal circuits uses specific neurotransmitters that bind to cognate receptors on other neurons. Genetic programs establish initial expression patterns of neurotransmitters in different classes of neurons (1–3), and activity-dependent neurotransmitter respecification modifies them during develop-

ment, either adding or switching transmitters (4–9). It is unknown, however, whether sensory stimuli promote transmitter switching in addition to other neuroplastic changes (10) in the adult brain. Alterations in photoperiod, circadian rhythm, and light exposure can each cause anxiogenic and depressive behavior in diurnal adult mam-

mals (11, 12). We hypothesized that nocturnal mammals would respond to light manipulation in the opposite manner (13) and that these changes in behavior would be mediated by transmitter switching. To test this hypothesis, we exposed adult rats to different photoperiods and determined whether transmitter specification and behavior were affected.

Photoperiod-Dependence of Dopamine Expression

The number of dopaminergic neurons in hypothalamic nuclei innervated by the retino-hypothalamic projection via the suprachiasmatic nucleus (SCN) (fig. S1) (14, 15) changed with the light-dark (L-D) cycle to which animals were exposed. Animals were maintained in photoperiod chambers on long-day [19 hours light:5 hours dark (19L:5D)],

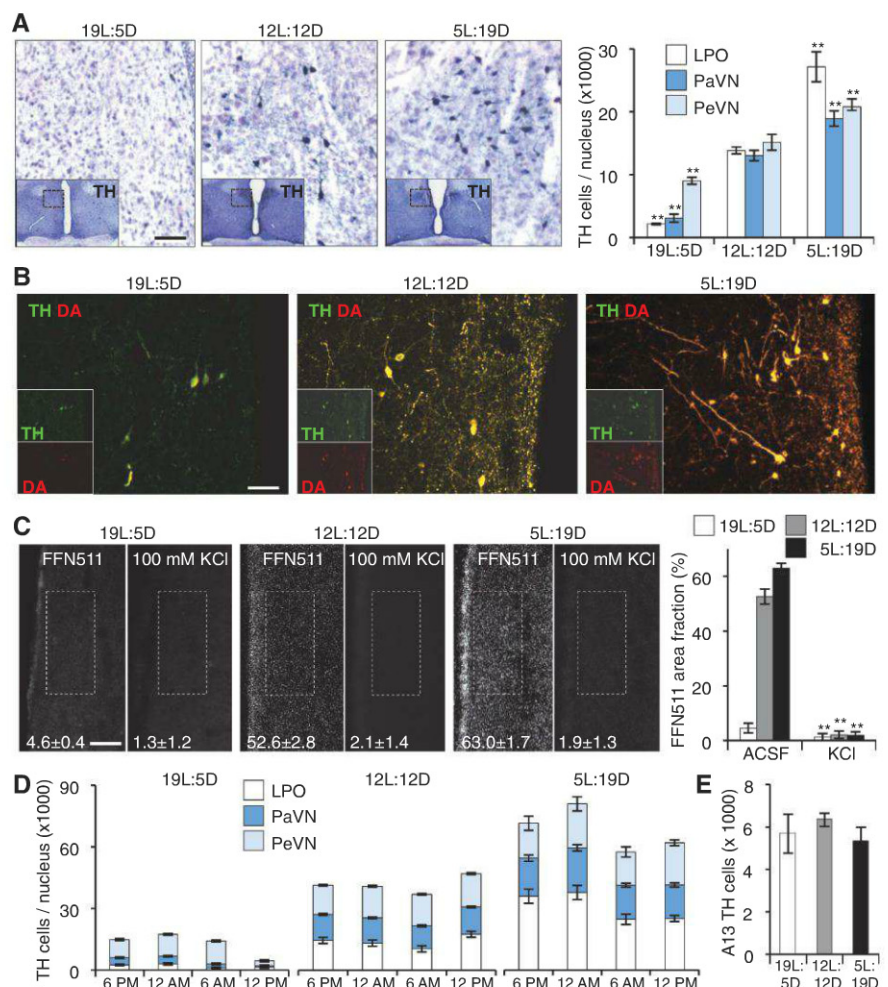
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Fig. 1. Photoperiods regulate numbers of dopaminergic neurons in hypothalamic nuclei. (A) Exposure to 19L:5D or 5L:19D photoperiods for 1 week changes the number of tyrosine hydroxylase immunoreactive (TH) neurons in the PaVN relative to control (12L:12D) (left). Number of TH neurons in the LPO, PaVN and PeVN for each condition (right). *n* = 12 animals for each photoperiod. (B) Dopamine is colocalized with TH in the PaVN after exposure to each of the photoperiods. *n* = 5 animals for each photoperiod. (C) FFN511 is taken up in the PeVN and released by KCl after exposure to each of the photoperiods (left). Fluorescence measured in boxed regions (right). *n* = 6 animals for each photoperiod. (D) Circadian fluctuations do not account for changes produced by different photoperiods. Numbers of TH neurons for all three nuclei at all four times from 19L:5D and 5L:19D are significantly different from those from 12L:12D. *n* = 5 animals for each photoperiod. (E) The number of TH neurons in the nearby A13 nucleus is not significantly different after exposure to each of the photoperiods. *n* = 5 animals for each photoperiod. ***P* < 0.01. Scale bars, (A) 120 μ m; (B) 100 μ m; (C) 80 μ m.



short-day (5L:19D), or balanced-day (12L:12D; control) cycles for 1 week, and sections through the lateral preoptic area (LPO), paraventricular nucleus (PaVN), and periventricular nucleus (PeVN) were immunostained for tyrosine hydroxylase (TH), an enzyme in the dopamine synthetic pathway (16). The number of TH-immunoreactive (TH-IR) neurons decreased with long-day exposure and increased with short-day exposure in relation to control (Fig. 1A and fig. S2). TH and dopamine were colocalized in all three conditions (Fig. 1B). To assess the functional status of TH-IR neurons, we examined uptake and release of fluorescent false neurotransmitter 511 (FFN511) (17), which is taken up by vesicular monoamine transporter 2 (VMAT2). FFN511 generated fluorescent signals in hypothalamic slices from brains of animals exposed to each of the three photoperiods. Fluorescence decreased upon KCl depolarization (Fig. 1C).

Neurotransmitter respecification could be induced through changes in photoperiod or alteration of the circadian rhythm. To address the circadian contribution to changes in numbers of TH-IR neurons, we quantified their numbers at 6-hour intervals in animals exposed to each of the different photoperiods for 1 week (Fig. 1D). Although there is variation at some time points during each photoperiod (table S1), values for all three nuclei at all four times from 19L:5D and 5L:19D were significantly different from

those from 12L:12D photoperiod exposure (table S2). The effects of different photoperiods are thus unlikely to have arisen from measurements collected at nonequivalent phases of the circadian rhythm. Week-long exposure to each of the different photoperiods failed to produce changes in numbers of TH-IR neurons in an adjacent nucleus, A13 (Fig. 1E and fig. S2B), which does not receive retinal input via the SCN (18).

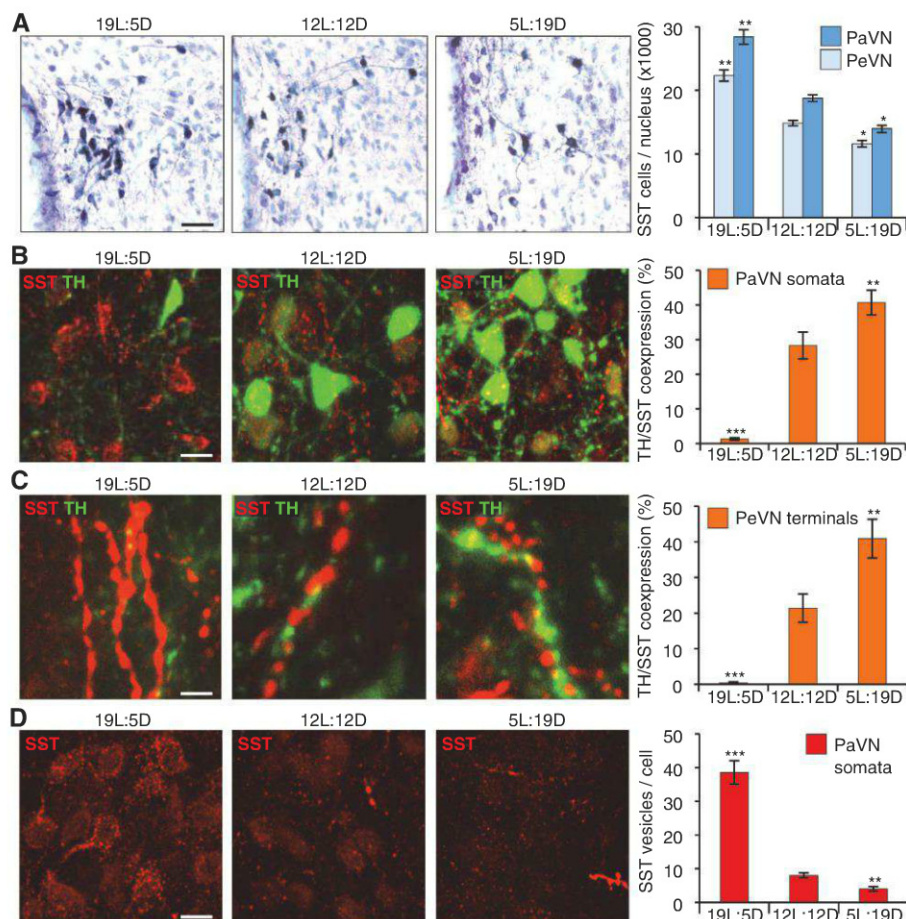
Transmitter Switching Between Dopamine and Somatostatin

Neurons in the PaVN and PeVN also express somatostatin (SST) (19, 20). The number of SST neurons showed an inverse relationship to the number of TH neurons with exposure to the three different photoperiods (Fig. 2A). This result could arise from changes in the numbers of neurons of each type or from transmitter switching within neurons. To determine whether these reciprocal changes result from adult neurogenesis (21, 22), we injected animals with BrdU on days 2 to 4 of the week of exposure. No significant BrdU labeling was detected in the LPO, PaVN, or PeVN (fig. S3A). To ascertain whether apoptosis causes decreased numbers of TH-IR neurons and SST-IR neurons after exposure to long- and short-day photoperiods, respectively, we subjected sections from animals after 1 week of exposure to each of the photoperiods to terminal deoxynucleotidyl

transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay. No significant differences in sporadic cell death were observed (fig. S3B). Photoperiod-dependent changes in the numbers of TH-IR neurons were reversible. When animals were exposed to 2 weeks of consecutive but opposite, short-, or long-day, photoperiods, TH expression did not differ from controls exposed to the 12L:12D photoperiod for 2 weeks (fig. S3C).

Are individual neurons reciprocally shifting transmitter expression between dopamine and SST? Double immunofluorescence revealed that different photoperiods changed the balance of dopamine and SST coexpression in neurons in the PaVN and PeVN. Long-day exposure reduced TH-IR and increased SST-IR, whereas short-day exposure increased TH-IR and decreased SST-IR (Fig. 2B). The 43% increase and 96% decrease in TH-IR/SST-IR coexpression after short- and long-day exposure, in contrast to the balanced photoperiod, suggest that TH-IR and SST-IR neurons are recruited from a reserve pool of cells (23) that are switching transmitters. Changes in TH and SST expression in cell bodies were also observed at high magnification in PaVN axons and en passant terminals in the periventricular region (Fig. 2C) and in PeVN cell bodies (fig. S4). The number of intracellular SST-IR storage vesicles depended on photoperiod light-cycle duration

Fig. 2. Photoperiods drive neurotransmitter switching between dopamine and somatostatin. (A) Exposure to 19L:5D or 5L:19D photoperiods changes the number of SST-IR neurons in the PaVN relative to control (12L:12D) (left). Number of SST neurons in the PaVN and PeVN for each photoperiod (right). $n = 6$ animals for each photoperiod. (B and C) SST and TH immunofluorescence in the (B) PaVN and (C) PeVN change after exposure to each of the photoperiods (left). TH/SST coexpression (right). $n = 7$ animals for each photoperiod. (D) The number of SST vesicles/neuron cell body in the PaVN follows photoperiod light cycle duration (left). $n = 9$ cells for each photoperiod. Scale bars, (A) 100 μm ; (B) 20 μm ; (C) 3 μm ; (D) 20 μm . * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.



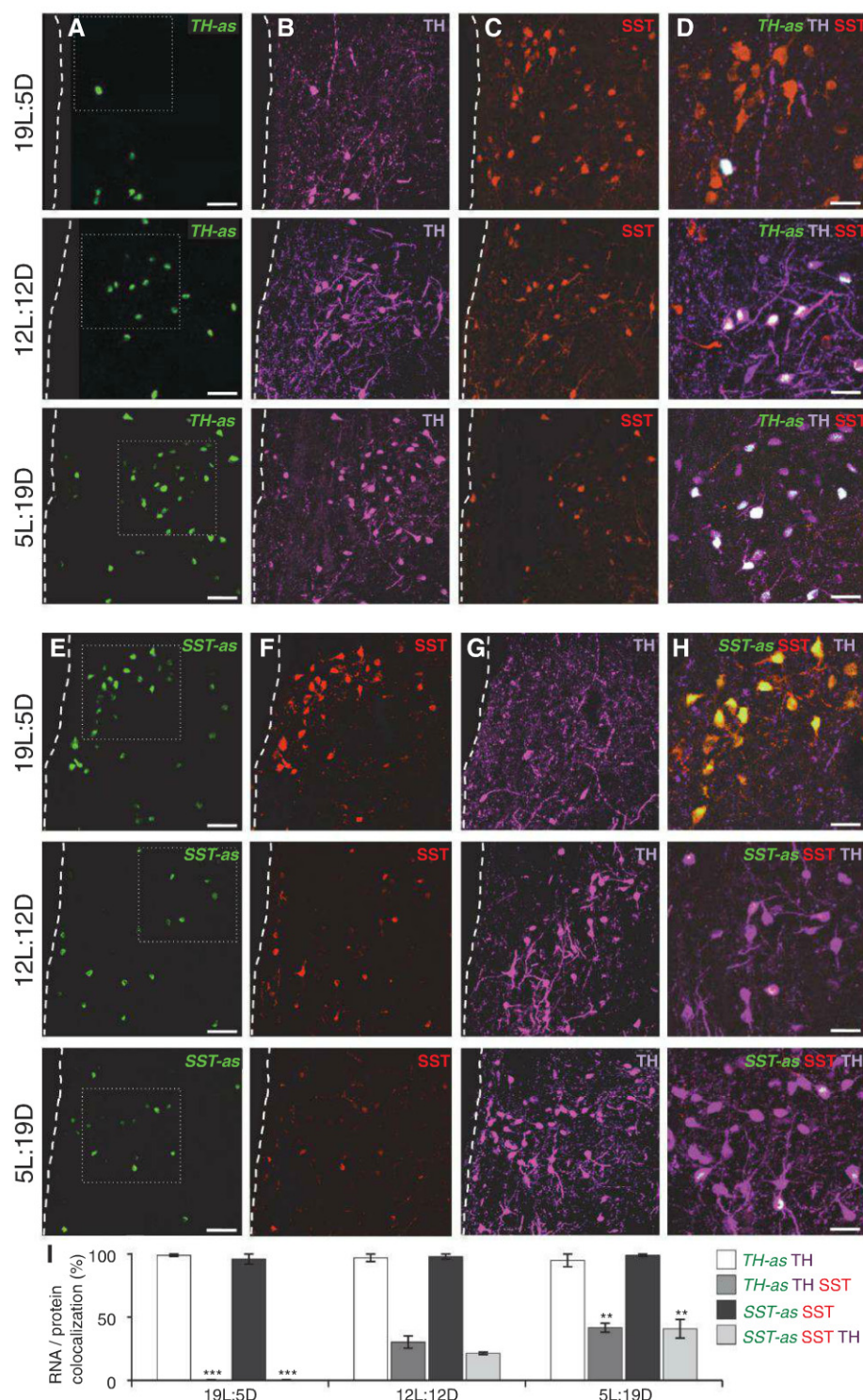


Fig. 3. Photoperiod-dependent changes in TH and SST parallel changes in TH and SST transcripts in the PaVN. Triple labeling of TH-antisense (*TH-as*) or *SST-as*, TH, and SST in single sections from animals exposed to each photoperiod. (A to C) Short-day photoperiods drive increases in numbers of *TH-as* and TH cells and a decrease in the number of SST cells. (D) Higher-magnification three-channel merged images of boxed regions at left. (E to G) Short-day photoperiods drive decreases in numbers of *SST-as* and SST cells and an increase in the number of TH cells. (H) Higher-magnification three-channel merged images of boxed regions at left. (I) More than 200 cells/PaVN were analyzed for each probe, and cells that were positive for one probe or the other were scored for colocalization with protein. Scale bars, (A) to (C) and (E) to (G) 100 μ m; (D) and (H) 50 μ m. $n = 8$ animals for (A) to (D) and (E) to (H); quantification (I) for all 16 animals.

(Fig. 2D). Decreased and increased numbers of TH-IR neuronal cell bodies resulting from exposure to long- and short-day photoperiods (Fig. 1A) were matched by corresponding increases and decreases in numbers of SST-IR neuronal cell bodies (Fig. 2A and fig. S5). Newly expressing TH-IR neurons induced through short-day photoperiod exposure coexpressed additional dopaminergic markers (3), VMAT2 (fig. S6), and the dopamine transporter, DAT (fig. S7).

To test the transcriptional dependence of transmitter respecification, we performed single-molecule fluorescence in situ hybridization with probes for TH and SST. Photoperiod-dependent changes in the number of labeled neurons identified regulation at the transcriptional level (Fig. 3, A to I), which is consistent with increased SST transcripts in PeVN neurons after exposure to stressors (24). Simultaneous immunostaining showed that changes in numbers of TH (Fig. 3, B and G) and SST (Fig. 3, C and F) neurons paralleled changes in mRNA expression (Fig. 3, D, H, and I). These results demonstrate that transmitter respecification is not achieved by translation from preexisting transcripts and involves de novo induction of TH or SST mRNA. Not all neurons underwent this transmitter switch; after exposure to the long-day photoperiod, 25% of the number of 12L:12D TH-IR PaVN neurons (Fig. 1A) expressed TH immunoreactivity without detectable SST immunoreactivity (Fig. 2B). SST was not expressed in the LPO after exposure to any of the three photoperiods, and a transmitter switch partner for dopaminergic neurons in this nucleus remains to be determined.

Changes in Postsynaptic Receptor Populations

Are presynaptic changes in transmitter identity matched by changes in postsynaptic receptor populations? SST interneurons of the parvocellular PaVN and the anterior PeVN make synapses on corticotropin-releasing factor (CRF) neurons distributed along the third ventricle, as do dopaminergic interneurons (25, 26). To determine whether dopamine D2 receptor (D2R) and SST were coexpressed on periventricular CRF cells innervated by TH-IR neurons, we stained sections with antibodies for CRF, SST2/4R, and D2R (Fig. 4A) (27, 28). After exposure to the control 12L:12D photoperiod, the two classes of receptors appeared colocalized on CRF cells along the ependymal layer in the PeVN, which is consistent with presynaptic transmitter coexpression (Figs. 2 and 3). After the long-day photoperiod, colocalization of D2R and SST2/4R was rare because D2R expression was down-regulated in parallel with the decrease in number of dopaminergic neurons. In contrast, after short-day photoperiod exposure, colocalization of these receptors was abundant as the number of dopaminergic neurons expanded (Fig. 4, A to C, and fig. S5).

We hypothesized that levels of CRF in the cerebrospinal fluid (CSF) would decrease as a result of increased inhibition resulting from exposure to the short-day photoperiod that leads to

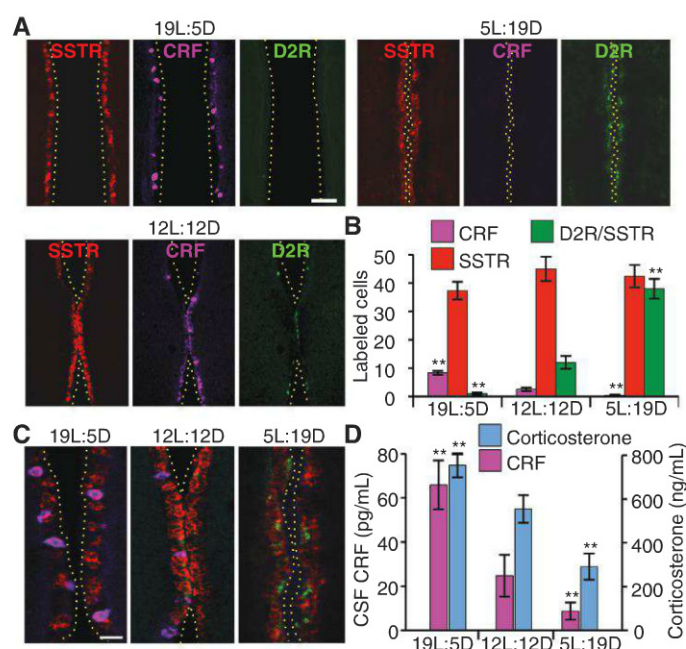
coexpression of D2R with SST2/4R, if receptor activation is more substantial than the level of presynaptic SST. We envisaged that levels would increase with reduced D2R expression after the long-day photoperiod. Assays of CRF in the CSF and corticosterone in the plasma confirmed this to be the case (Fig. 4D). The number of CRF-immunoreactive neurons was also reduced after exposure to the short-day photoperiod (Fig. 4, A to C), reflecting low levels of CRF in the absence of apoptosis and consistent with reduced induction of CRF synthesis due to increased inhibition by both D2R and SST2/4R (29, 30). SST2/4R but not D2R expression was observed on CRF neurons in the PaVN.

Photoperiod-Dependence of Behaviors

To investigate behavioral consequences of these changes in transmitter status, we tested rats on the elevated plus maze (EPM) (31) and in the forced swim test (FST) (32, 33). Relative to controls, short-day exposure caused rats to spend more time exploring the open arm of the maze (movies S1 and S2) and spend a longer time swimming before becoming immobile (movies S3 and S4). Locomotor behavior and swimming ability appeared normal. Long-day exposure produced the opposite effects (Fig. 5A). The results of EPM testing during the middle of the light or dark phases after 24-hour-cycle photoperiods were not different. EPM testing at the conclusion of treatment with the same 19L:5D, 12D:12L, or 5L:19D ratios of total light/dark exposure distributed across much shorter (72 min) cycles yielded indistinguishable results (fig. S8).

To determine whether the behavioral changes observed are specifically related to changes in the number of TH-IR neurons, we made stereotaxic injections of 6-hydroxydopamine (6-OHDA) adjacent to the PaVN (fig. S9, A and B) so as to focally ablate TH-IR neurons in animals maintained on a 12L:12D cycle. Artificial CSF (ACSF) was injected as a sham control. After ablation, we observed a 95% reduction in time spent exploring the open arm of the EPM and a 57% increase in duration of the immobility time in the FST (Fig. 5B). Infusion of animals on a 12L:12D cycle with dopamine receptor antagonists SCH23390 and sulpiride, which selectively block D1 and D2 dopamine receptors (34), affected these behaviors similarly to 19L:5D exposure (Fig. 5C). Neither 6-OHDA nor receptor antagonists affected locomotor activity or swimming performance. Post-test histology of 6-OHDA-injected animals revealed that the number of TH-IR neurons was decreased by more than 50% in all three of these hypothalamic dopaminergic nuclei (Fig. 5D). The total number of cells in the PaVN was reduced (fig. S9C), indicating loss of neurons after 6-OHDA treatment. However, counts of TH-IR neurons in A13 after 6-OHDA treatment were not affected, demonstrating that dopaminergic nuclei located as close as 200 μ m were spared by focal toxin delivery (Fig. 5D). Sham operation and injection of ACSF had no effect on the number of TH-IR neurons.

Fig. 4. Dopamine and somatostatin receptor colocalization on CRF neurons. (A) Triple labeling of SST2/4R, CRF, and D2R in single sections after exposure to each photoperiod. (B and C) D2R and SST2/4R colocalization on CRF cells. Labeled cells were scored along the walls of the third ventricle (dotted lines), and the number was averaged for 10 30- μ m sections of the rostral hypothalamus. For (B), $n = 6$ animals. (D) CRF levels in the CSF and corticosterone levels in the plasma after exposure to each photoperiod. $n = 6$ animals. $**P < 0.01$. Scale bars, (A) 120 μ m; (C) 40 μ m.



Role of Newly Dopaminergic Neurons in Stress Behaviors

We next determined whether photoperiod-dependent hypothalamic transmitter respecification reported here was likely to account for the observed changes in stress responses. We first ablated TH-IR neurons in these nuclei by means of local stereotaxic injection of 6-OHDA and then exposed animals to the long-day photoperiod, so as to further reduce their number, or to the short-day photoperiod, so as to recruit more neurons to become TH-IR (fig. S10). The behavioral results of focal ablation of TH-IR neurons were partially reversed by exposure to the short-day photoperiod, demonstrating behavioral rescue (Fig. 5E). Post-test histology demonstrated photoperiod-dependent reduction and induction of TH-IR neurons (Fig. 5F). The appearance of newly dopaminergic neurons is unlikely to have resulted from recruitment from a preexisting dopaminergic pool of neurons with undetectable levels of neurotransmitter because the inducible reserve pool was not ablated by 6-OHDA before exposure to the short-day photoperiod. FPN511 uptake and release in hypothalamic slices confirmed the functionality of newly dopaminergic neurons (fig. S11).

Because behavioral recovery could be achieved by a parallel mechanism not involving recruitment of newly TH-IR neurons in these nuclei, we determined whether it could be reversed by acute, local infusion of dopamine receptor antagonists. TH-IR neurons were again ablated with 6-OHDA, and animals were exposed to the short-day photoperiod for 1 week to recruit newly TH-IR neurons. ACSF or SCH23390 and sulpiride were stereotactically and acutely introduced through a cannula into the region in between the LPO and PaVN. Behavioral testing showed decreased time in the open arm of the EPM and increased immobility time in the FST in response to infusion

with antagonists as compared with infusion with ACSF (Fig. 5E).

Discussion

Our results demonstrate transmitter switching between dopamine and somatostatin in neurons in the adult rat brain, induced by exposure to short- and long-day photoperiods that mimic seasonal changes at high latitudes. The shifts in SST/dopamine expression are regulated at the transcriptional level, are matched by parallel changes in postsynaptic D2R/SST2/4R expression, and have pronounced effects on behavior. SST-IR/TH-IR local interneurons synapse on CRF-releasing cells, providing a mechanism by which the brain of nocturnal rats generates a stress response to a long-day photoperiod, contributing to depression (35, 36) and serving as functional integrators at the interface of sensory and neuroendocrine responses (36–38).

SST/dopamine interchange illustrates neuroplasticity in self-balancing networks (39) that tunes neural function and behavior to a dynamic environment. The molecular mechanism remains to be discovered, but AST-1 and Etv1 transcription factors bind to a specific dopamine motif in *Caenorhabditis elegans* and mouse to drive the expression of genes that encode components necessary for synthesis, packaging, and reuptake of dopamine (3, 40). Activity-dependent transmitter switching may serve functions similar to homeostatic synaptic scaling (41), changes in ion channel expression (42), and neuropeptide remodeling of sensory networks (43) and is an additional form of brain plasticity to add to modifications of synaptic strength, synapse number, and electrical excitability. Sensory stimulation driving transmitter switching in specific microcircuits affected in neurological or psychiatric disorders could contribute to noninvasive restoration of normal function in the mature nervous system.

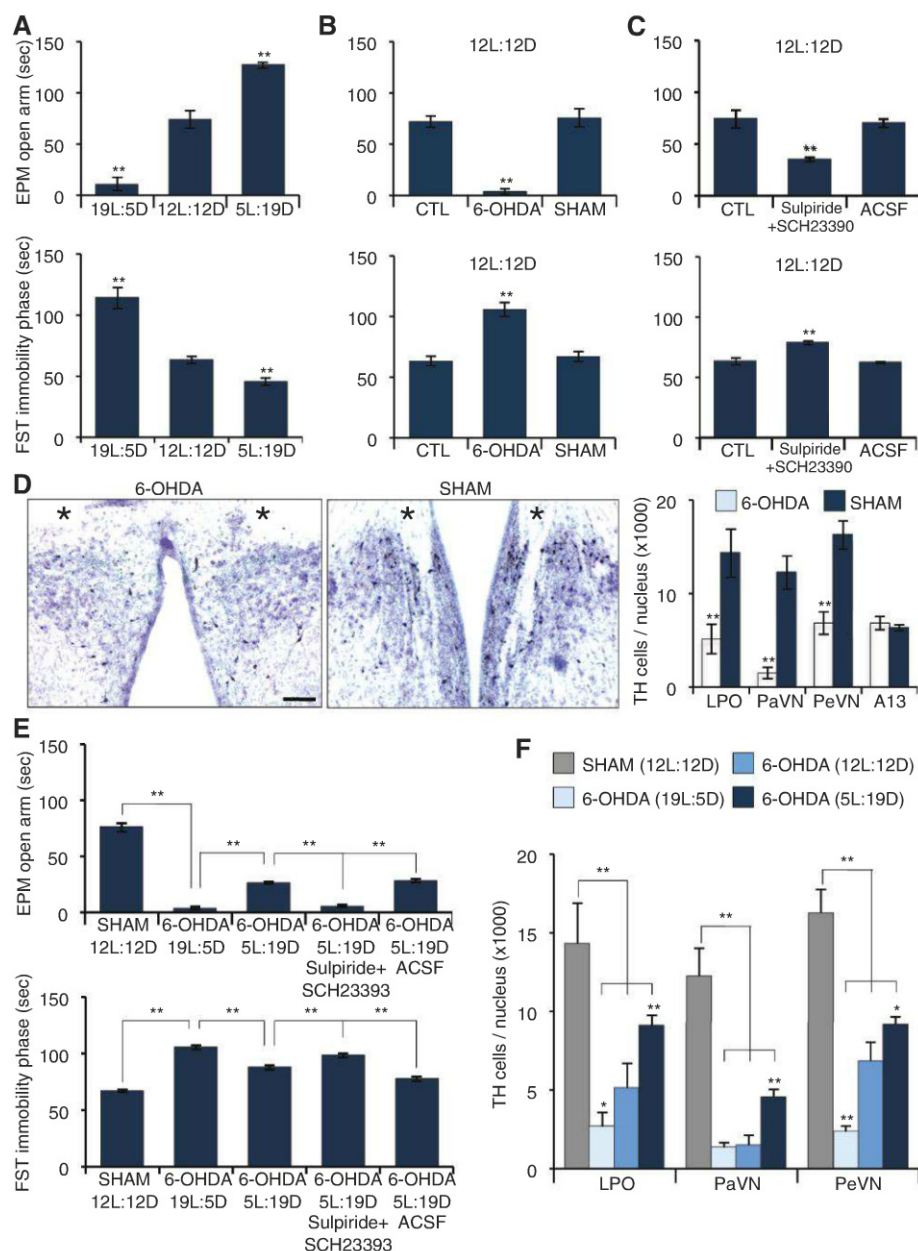


Fig. 5. Behavioral changes after lesions of dopaminergic neurons are rescued through photo-period induction of newly dopaminergic neurons. (A) Baseline behavior on EPM and FST after exposure to 19L:5D, 12L:12D, or 5L:19D photoperiods for 1 week. (B) Effect of 6-OHDA ablation of DA neurons versus sham operation on behavior of animals exposed to 12L:12D for 1 week. (C) Behavior of 12L:12D animals without and with acute infusion of D1 and D2 receptor antagonists, SCH23390 and sulpiride. (D) Histology of tissue and stereology of TH-IR neurons after 6-OHDA, accompanying behavioral tests. Asterisks indicate injection sites. Scale bar, 150 μ m. For (A) and (C), $n = 5$ animals; for (B) and (D) $n = 10$ animals. $**P < 0.01$ compared with 12L:12D control [(A) to (C)] and to sham (D). (E) Effect on behavior of 19L:5D or 5L:19D exposure for 1 week after 6-OHDA ablation of DA neurons in animals exposed to 12L:12D. The rescue achieved with 5L:19D exposure is selectively abolished by infusion of D1 and D2 receptor antagonists, SCH23390 and sulpiride. (F) Number of TH neurons after 6-OHDA ablation of LPO, PaVN, and PeVN in animals exposed to 12L:12D followed by 19L:5D, 12L:12D, or 5L:19D for 1 week. For (E) and (F), $n = 5$ and 4 animals, respectively. $*P < 0.05$. $**P < 0.01$ for (E) comparisons between adjacent groups and (F) comparisons with 6-OHDA [sham (12L:12D)].

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Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6131/449/DC1
Materials and Methods
Figs. S1 to S11
Tables S1 and S2
Movies S1 to S4

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Vaterite Crystals Contain Two Interspersed Crystal Structures

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Calcite, aragonite, and vaterite are the three anhydrous polymorphs of calcium carbonate, in order of decreasing thermodynamic stability. Although vaterite is not commonly found in geological settings, it is an important precursor in several carbonate-forming systems and can be found in biological settings. Because of difficulties in obtaining large, pure, single crystals, the crystal structure of vaterite has been elusive for almost a century. Using aberration-corrected high-resolution transmission electron microscopy, we found that vaterite is actually composed of at least two different crystallographic structures that coexist within a pseudo-single crystal. The major structure exhibits hexagonal symmetry; the minor structure, existing as nanodomains within the major matrix, is still unknown.

By far the most abundant biogenic minerals are calcium carbonates, which exist in different polymorphs: calcite, aragonite, vaterite, and amorphous CaCO_3 , listed in order of decreasing thermodynamic stability under normal conditions. There are numerous examples of biogenic calcite and aragonite, as well as a growing list of discoveries of biogenic CaCO_3 formation via transient amorphous calcium carbonate (1–3). Vaterite is much rarer as a geologic mineral or biomineral, possibly because of its instability. Vaterite is an important constituent of Portland cement (4) and has been found during oil field drilling (4). It has also been detected in gallstones (5) and even as part of a meteorite (6). Synthetic vaterite is common in various scenarios, such as under template-directed (7, 8) or additive-assisted (9) conditions.

In biominerals, vaterite occurs as a minority component of a larger formed structure or as the result of a defective biological process. Some examples can be found in green turtle eggshells (10), in coho salmon otoliths (11), in freshwater lackluster pearls (12), and in the aberrant growth of mollusk shells repaired after an injury (13). A rare example of deposition of vaterite as the only mineral component of an organism's endoskeleton can be found in the body and tunic spicules of the solitary stolidobranch ascidian *Herdmania momus* (14) (Fig. 1).

Gibson (15) showed that the x-ray diffraction pattern of vaterite is distinct from that of calcite or aragonite. The first symmetry that was related to vaterite was the hexagonal symmetry

described by Olshausen in 1925 (16–18). More than 30 years later, Meyer (19) provided the first full crystallographic description (atomic positions) of vaterite, describing its unit cell as having pseudo-hexagonal-orthorhombic symmetry. The next model for vaterite, which was proposed in 1963 by Kamhi (17) and has become the most widely accredited structure, posits 100% occupancy by the calcium and one-third occupancy by the carbonate groups. Notably, Kamhi also reported eight weak reflections that could not be indexed, and attributed them to a superstructure, rotated 30° about the c axis from the main one (Kamhi's hexagonal structure), with lattice parameters $a = \sqrt{3}a'$ and $c = 2c'$. Meyer (20) suggested another structure in which the basic unit cell was essentially in agreement with Kamhi's model but the site symmetry of the carbonate groups was different. Meyer also emphasized that the observed additional spectral features (diffuse streaks along the c axis, and satellite reflections) were attributable to stacking faults perpendicular to the c axis, leading to doubling or tripling of the c lattice parameter.

The principal debate has centered on whether the vaterite symmetry is hexagonal or orthorhombic, although the Raman spectrum of vaterite did not agree with either of the proposed structures (21). Wang and Becker (22), using density functional theory (DFT), proposed a new unit cell with the $P6_322$ space group. Recently, Le Bail (23) suggested a modified orthorhombic unit cell based on a microtwinning hypothesis. Demichelis *et al.* (24), also using DFT, took energy considerations into account, and this led to new vaterite structures that have greater stability in simulations. Interestingly, Demichelis *et al.* reported several theoretically stable structures that required only room-temperature thermal energy for their interconversions. They suggested that these frequent interconversions provide an additional source of disorder within the vaterite structure. Mugnaioli *et al.* (25), using automated diffraction tomography (ADT) and precession

electron diffraction on a transmission electron microscope, suggested two structures with very low symmetries: a monoclinic unit cell and a triclinic one. To account for all the diffraction data obtained in the studies of Mugnaioli *et al.*, almost the lowest symmetries have been hypothesized (25).

We used high-resolution synchrotron powder diffraction and aberration-corrected high-resolution transmission electron microscopy (HRTEM) to examine the structure of vaterite, using the vaterite spicules of *H. momus* ascidians as samples. Relative to the synthetic vaterite used in previous studies, the single crystals in these spicules are larger and have fewer lattice defects. The spectrum obtained from high-resolution synchrotron powder diffraction on beamline ID31 of the European Synchrotron Radiation Facility (ESRF), combined with Rietveld refinement, could not be fitted by any of the structures proposed for vaterite. The hexagonal structure proposed by Kamhi (17) gave the best fit to the data but did not explain all the experimentally observed diffraction peaks (fig. S1).

We then used HRTEM investigations of *H. momus* spicules to study very small volumes of vaterite, so as to ensure that data would not come from different structures. An additional advantage of using these spicules is that they are perfectly and reproducibly oriented. When indexed in the hexagonal setting, each thorn on the tunic spicule comprises a single crystal of vaterite, which grows precisely along the c axis (Fig. 2A). This allowed us to prepare TEM specimens by cutting with a focused ion beam each single crystal precisely along known crystallographic directions. By cutting the thorns parallel, perpendicular, or at other known angles to the main axis, we could study the vaterite structure along different zone axes.

Analysis of all the electron diffraction patterns collected at different zone axes (Fig. 2) shows that each thorn diffracted as a single crystal of vaterite. Furthermore, the corresponding phase-contrast images demonstrated perfect crystalline order. In Fig. 2C, an array of strong diffraction spots, as well as weaker ones, can be

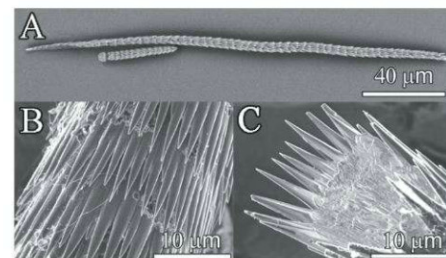


Fig. 1. SEM images of the *Herdmania momus* vaterite spicules. (A) A body spicule (long) and a tunic spicule (short). (B and C) Two different observation directions of two tunic spicules and their crowns of thorns (acceleration voltage 4 kV, in-lens detector).

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observed. When we used the JEMS package (26) to simulate electron diffraction patterns and high-resolution images, it was clear that the strong

diffraction spots in Fig. 2C could not be fully explained by a single structural model (fig. S2 and table S1). Although both the Kamhi and

Meyer models combined were needed to explain the strong diffraction spots, neither model could explain any of the weak diffraction spots. Conversely, along the other zone axis (Fig. 2A), all diffraction spots in Fig. 2E could be explained by the Kamhi model, whereas the Meyer model could explain only some of them. Using JEMS, we simulated each of the proposed models for vaterite structure, and found that none of them could explain all the HRTEM diffraction spots.

In seeking a solution to this conundrum, we examined additional zone axes of vaterite with the thought that these might reveal additional information. A zone axis perpendicular to the *c* axis (in the hexagonal setting) is shown in fig. S3. In this third zone axis, both the diffraction and the phase-contrast images revealed, perpendicular to the *c* axis, planar defects that were predominantly stacking faults. These observations were in agreement with Kamhi's report of diffuse scattering perpendicular to the *c* axis (17). However, when looking at different areas of this phase-contrast image, we observed distinct areas with variable atomic ordering (Fig. 3, A and B), indicating the possibility that the sample was not in fact a single crystal. Fast Fourier transform (FFT) analysis of the part of the phase-contrast image that includes the planar defects yielded a pattern that could be fitted to the electron diffraction (Fig. 3, C and E) of the same zone axis. This FFT-generated diffraction-like pattern also showed a good fit to the Kamhi model. However, when FFT analysis was performed only on the area containing the distinctly different atomic structure from the selected area observed in Fig. 3B (free of planar defects), we found that it did not match the FFT pattern generated from the other area depicted in Fig. 3A (see Fig. 3F) and showed no correlation with the Kamhi model or with any other model we examined.

For additional verification, we acquired electron diffraction patterns using an electron beam with a diameter of only 3 to 4 nm. Changes in diffraction patterns from different positions within a single thorn were observed. In diffraction images obtained from areas that could be seen to be distinct in the phase image, such as in Fig. 3B, we observed that the strong diffraction pattern that fit the Kamhi structure was now considerably weakened and that new diffraction points from a different structure were visible (compare Fig. 3D and Fig. 3C). The latter observation demonstrates that within a "single crystal" of vaterite there are at least two distinct coexisting structures. Even the diffraction pattern observed in Fig. 3C, taken from the selective area in Fig. 3A, is a superposition of both structures, but because of the large proportion of the Kamhi structure relative to the minor structure, the diffraction spots from the latter are not observed.

This observation was also consistent over several different samples cut from the same spicule, and from different tunic spicules. Upon examining the same [210] zone axis in each of these samples, we observed that the main structure,

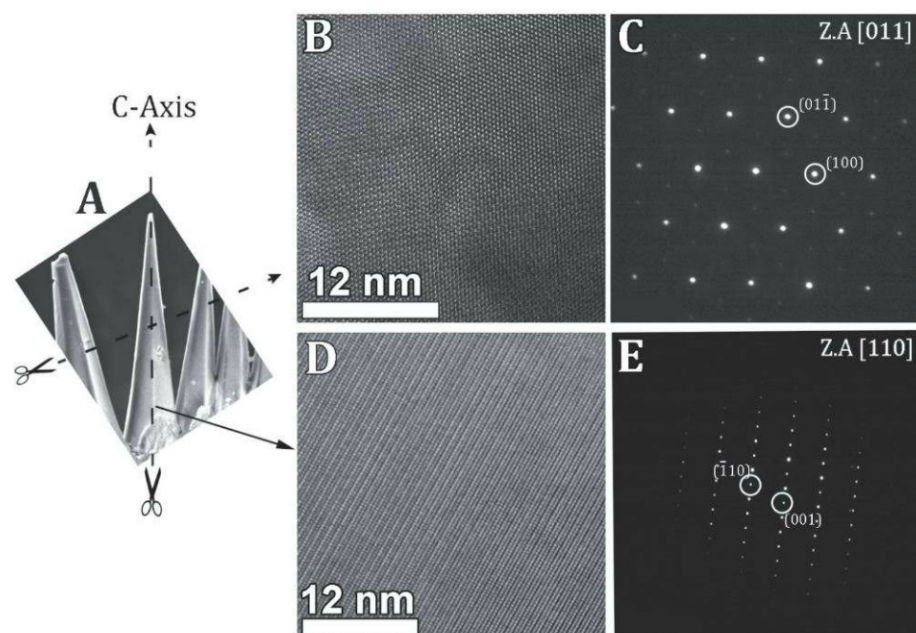


Fig. 2. HRTEM images (acceleration voltage 300 kV) and electron diffractions (acceleration voltage 200 kV) in two different zone axes. (A) Schematic illustration of the focused ion beam cutting direction on vaterite crystals from a tunic spicule of *H. momus*. The electron beam in TEM is perpendicular to the cutting direction. (B to E) Different phase-contrast images [(B) and (D)] and their respective corresponding electron diffractions [(C) and (E)]. The images in (B) and (C) were produced from a sample cut at $\sim 70^\circ$ to the long axis of a single thorn; those in (D) and (E) were produced from a sample cut parallel to the long axis of a single thorn.

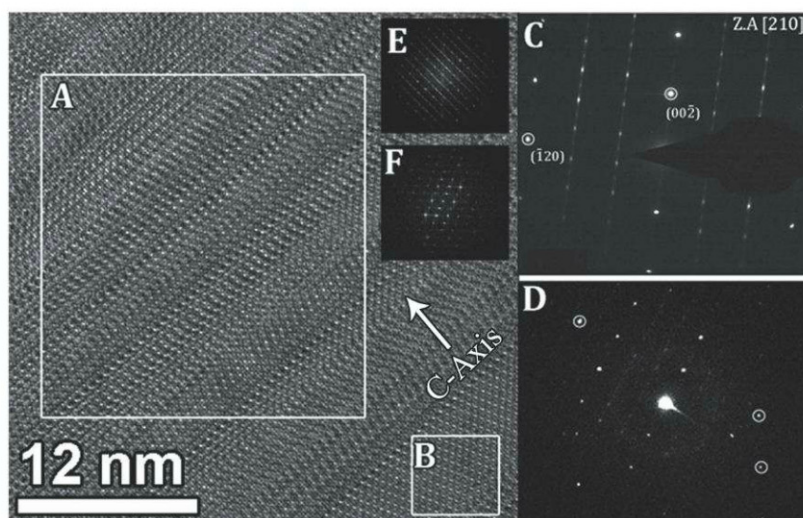


Fig. 3. HRTEM phase contrast image (acceleration voltage 300 kV) produced from a sample cut parallel to the long axis of a single thorn. A few distinct areas and their corresponding electron diffractions and FFT patterns can be seen. (A) Stacking faults. Despite the stacking faults, the diffraction pattern is in good agreement with the Kamhi structure. (B) Distinct atomic structure with no planar defects. (C) Electron diffraction corresponding to zone axis [210] taken from (A). (D) Microdiffraction taken from (B), showing superposition of two electron diffraction patterns from the main (Kamhi) structure (zone axis remains unaltered [210]) and an additional, distinct one (which seems not to be in the zone axis). Gamma correction was performed, but the quantitative analysis was not based on spot intensity. A few diffraction spots from the minor distinct structure are circled. (E) FFT corresponding to (A) [providing an equivalent pattern to that of electron diffraction shown in (C)]. (F) FFT corresponding to (B).

despite the planar defects, was the Kamhi structure; the minor but clearly distinct structure is unknown. We did not observe an ordered array of these two structures, but rather an apparently random distribution.

The possibility that the observed phenomenon was induced by the focused ion beam can be ruled out. All samples prepared from the same “single crystal” showed what appeared to be a perfect single crystal along the c axis; in addition, the multiple-structure phenomenon was observed only along the specific zone axis perpendicular to the c axis. This was further verified when we prepared TEM samples with the same zone axis by means of bombardment with argon ions and observed the same structures (fig. S3).

Because the two distinct structures were observed only when imaging a sample tilted to a zone axis perpendicular to the main long axis of a single thorn (the c axis in the Kamhi model), we tried to image the structure by tilting to a zone axis parallel to the long axis of a single thorn, [001]. Although hardly any beam damage was observed at other orientations, samples tilted to the [001] zone axis were always sensitive to the electron beam, and we therefore had to work with the much lower acceleration voltage of 80 keV. We are not sure why this zone axis was the most vulnerable to the electron beam, but it seemed that intracrystalline organic macromolecules were situated mainly on planes perpendicular to the c axis (fig. S3). When the image obtained at low acceleration voltage was stable, it could be seen that although the structure diffracted as a single crystal, it was actually made up of smaller domains.

When two coexisting domains were observed, such as those seen in Fig. 4, it was evident that not only were they rotated by 30° from one another, but they also had distinct d -spacings,

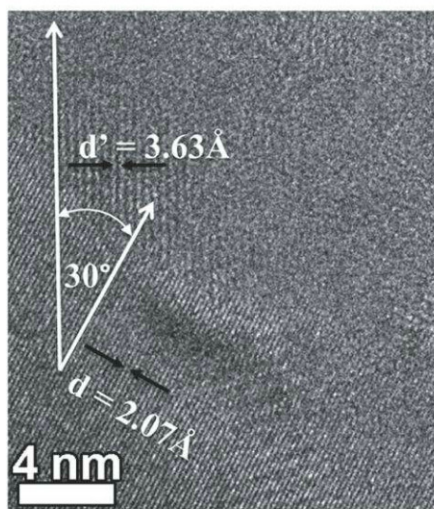


Fig. 4. HRTEM image (acceleration voltage 80 kV) with two lattices. The lower lattice has d -spacing = 2.07 ± 0.05 Å and the upper has d' -spacing = 3.63 ± 0.05 Å, and the two lattices are rotated by 30° .

intercorrelated by exactly $\sqrt{3}$. The d -spacing of the first domain showed a good fit to that of the Kamhi {110} d -spacing (2.07 ± 0.05 Å), whereas the d -spacing of the minor structure was 3.63 ± 0.05 Å. In his original paper on vaterite Kamhi observed, by means of single-crystal diffraction, eight weak diffraction points that he was not able to index, but he was able to report that the observed image resembled a supercell with a lattice rotated by 30° around the c axis and with lattice parameters of $a' = \sqrt{3}a$ and $c' = 2c$. Here, we instead observed two distinct crystalline structures that were not parts of a supercell but were completely different structures coexisting within a “single crystal” of vaterite. Clearly, the predominant structure was the Kamhi structure.

It is intriguing that both electron diffraction and phase contrast revealed what appeared to be perfect single crystals, and that the coexisting two structures were revealed only when the samples were tilted to the two zone axes ([210] and [001]). We stress that although all the various models suggested for vaterite have different unit cells, they all share common features: The carbonate ions are parallel to the c axis, and the calcium ions are organized in a hexagonal structure with similar inter-ion distances. Because TEM is much more sensitive to heavier atoms (calcium in vaterite) than to lighter atoms (oxygen and carbon), most of the contrast under standard conditions is derived from the calcium ions. If the calcium ions in the main and minor distinct structures we observed had matching organizations, it is unlikely that these two structures would be distinguishable in most crystallographic directions. It is therefore likely that the local calcium ion arrangement is conserved across the main and the minor structures, and that only the carbonates differ. This would produce a pseudo-epitaxial effect.

The finding that two distinct atomic structures coexist in a “single crystal” of vaterite may well explain the discrepancies and disputes generated by the experimental results and theoretical calculations published over the past 60 years (for data regarding the d -spacings derived from the FFT in Fig. 3F, and for comparisons with calcite and aragonite, see table S2). Inconsistencies among the published results are found not only in diffraction data but also in spectroscopic data such as Raman spectra and even solid-state nuclear magnetic resonance (27). In Raman spectra, for example, symmetric stretching vibration (ν_1) of the carbonates reveals a triplet in some studies and a doublet in others. The Kamhi structure predicts a doublet for this peak (21). In the seminal Raman study of vaterite by Wehrmeister *et al.* (21), it was concluded that the vaterite, owing to stacking of the carbonate groups, might possess a lower symmetry than that proposed by Kamhi or Meyer. Lower symmetry was also recently proposed by Mugnaioli *et al.* (25) who, in an elegant study based on ADT and other techniques, proposed two low-symmetry struc-

tures, one of which has triclinic symmetry (space group $P1$), which is almost the lowest symmetry possible. The electron beam diameter in that study was 50 nm. In our study we saw domains on a much smaller scale, only a few nanometers in diameter, in two different structures. We believe these two structures were not found by other researchers because they did not have the resolution we were able to achieve with the aberration-corrected HRTEM, and so they reported an average over the two structures. Even in the work of Mugnaioli *et al.* (25), where a 50-nm electron beam was used, what the authors observed was a superposition of the two structures.

Our findings indicate that the vaterite crystal structure is not a single entity; rather, vaterite consists predominantly of a hexagonal structure with at least one other coexisting crystallographic structure. The two structures are rotated by 30° with respect to one another and have lattice parameter relationships: $a' = \sqrt{3}a$. The challenge ahead is to fully characterize and solve the crystallographic structure of the minor structure and to understand how these two structures are organized relative to one another in space.

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Supplementary Materials

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Materials and Methods

Supplementary Text
Figs. S1 to S4
Tables S1 and S2
References (28–30)

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Direct Evidence of a Dinuclear Copper Intermediate in Cu(I)-Catalyzed Azide-Alkyne Cycloadditions

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Copper(I)-catalyzed azide-alkyne cycloaddition has become a commonly employed method for the synthesis of complex molecular architectures under challenging conditions. Despite the widespread use of copper-catalyzed cycloaddition reactions, the mechanism of these processes has remained difficult to establish due to the involvement of multiple equilibria between several reactive intermediates. Real-time monitoring of a representative cycloaddition process via heat-flow reaction calorimetry revealed that monomeric copper acetylide complexes are not reactive toward organic azides unless an exogenous copper catalyst is added. Furthermore, crossover experiments with an isotopically enriched exogenous copper source illustrated the stepwise nature of the carbon–nitrogen bond-forming events and the equivalence of the two copper atoms within the cycloaddition steps.

Rigorous examination of catalytic reaction mechanisms remains a formidable but necessary pursuit for organic chemists. In-depth reaction analysis can aid in the optimization of reaction parameters, spur the development of new catalytic processes, and enhance understanding of fundamental reactivity. The status quo for analysis of catalytic systems usually relies on one or a combination of the following methods: computational studies; isolation and reintroduction of an intermediate from the reaction mixture as proof of its involvement within the catalytic cycle; analysis of stereoelectronic effects of substituents present in the reactive intermediates; isotope crossover and kinetic isotope effects; and, most commonly, initial rate studies. Although these methods have shaped our current understanding of reaction mechanisms, they seldom provide a complete picture of complex catalytic reactions.

Among transition-metal-catalyzed processes, reactions involving copper remain particularly difficult for rigorous mechanistic investigation due to the low reduction potential of copper [$+0.159$ V from Cu^{2+} to Cu^+ and $+0.520$ V from Cu^+ to Cu^0 (1)], the propensity for Cu^+ species to disproportionate in solution (2), the generally poor ability of copper to backbond to ligands (3), and the well-documented tendency of copper complexes to aggregate (4). Despite these complications, copper catalysis has been em-

ployed successfully for decades in a variety of methods, including carbon–heteroatom bond formation (5); C–C bond formation (6); oxidative processes (7, 8); and, most recently, dipolar cycloadditions (Fig. 1A) (9–12). The latter of these includes the copper(I)-catalyzed azide-alkyne cy-

cloaddition (CuAAC), which has garnered widespread attention since its debut in 2001 as a facile and robust method for the creation of covalent linkages in a variety of environments. However, due to the aforementioned challenges in the direct study of copper catalysis, a mechanism of this reaction has yet to be fully elucidated; specifically, the identity and nuclearity of the copper species involved in catalysis has not been established (Fig. 1B). Herein, we propose from simple, deductive experiments a mechanism that necessitates two copper atoms within the active cycloaddition complex, and we describe their relation to one another.

Several studies toward elucidation of the mechanism of the CuAAC with terminal alkynes have been reported (13, 14), and experimental evidence of the possible involvement of polynuclear copper(I) intermediates (14–16) has been supported by theoretical studies (17, 18). Furthermore, recent advances in dipolar cycloadditions of 1-halo- and 1-metalloalkynes (formally internal alkynes) with organic azides have prompted additional investigation of the mechanism (19–22). In the case of 1-iodoalkynes, scission of the terminal alkyne-halogen bond to form a copper(I)-acetylide is not necessary for the cycloaddition

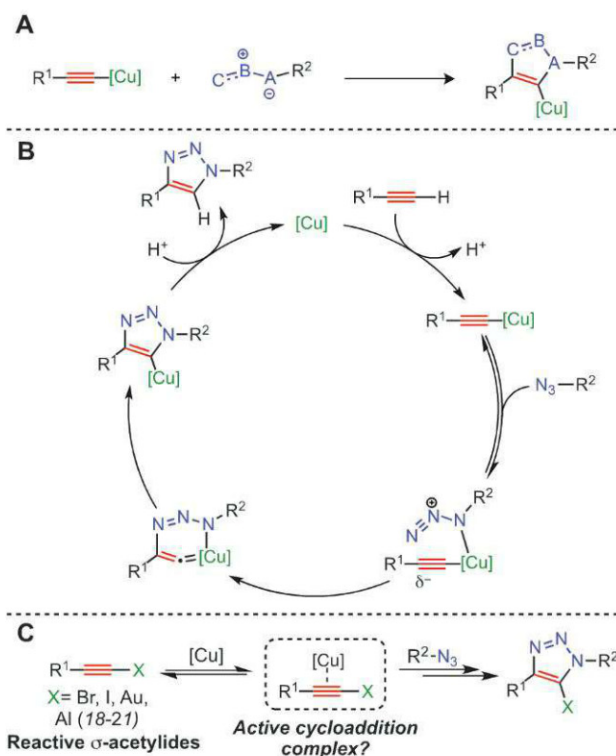


Fig. 1. CuAAC. (A) Generic form of the copper-catalyzed dipolar cycloaddition reaction. (B) First proposed mechanism of the CuAAC (10). (C) Copper-catalyzed cycloaddition of terminal and 1-iodoalkynes.

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to occur, suggesting that the copper catalyst effects the cycloaddition purely through weak π -interactions with the formally internal alkyne (Fig. 1C). Thus, we hypothesized that the classical CuAAC (with terminal alkynes) also proceeds through a similar reaction manifold, via: (i) in situ formation of the σ -bound copper acetylide; (ii) recruitment of a second π -bound copper atom, forming the catalytically active complex; (iii) reversible coordination of the organic azide to the π -bound copper complex; and (iv) the stepwise annulation events. Obtaining experimental support for this pathway would lead to a common mechanistic model involving activation of σ -acetylides (both terminal and formally internal) by weak and reversible π -interaction with a copper center (Fig. 1C).

Despite the multiple equilibria of unstable, non-isolable, and highly reactive intermediates within the catalytic cycle of the CuAAC, the copper(I)-acetylide and -triazolide intermediates can be reliably isolated and characterized (fig. S1) (23). Therefore, to investigate the mechanistic model proposed above, we envisioned a simple experiment in which a preformed, stable, mononuclear σ -bound copper(I)-acetylide is treated with a stoichiometric amount of an organic azide in the presence or absence of an added copper(I) catalyst, allowing us to directly verify the requirement of the putative π -bound copper intermediate. To this end, the σ -bound copper(I)-acetylide complex **2** was synthesized on a large scale (>20 g) in five overall steps (fig. S2), following the procedure reported by Nolte *et al.* (23). Subsequently, azide **1** was treated with acetylide **2** [0.090 and 0.082 M in tetrahydrofuran (THF), respectively] under two sets of conditions—with and without soluble exogenous copper catalyst **3** [$\text{Cu}(\text{PPh}_3)_2\text{NO}_3$, 0.0041 M; Ph, phenyl]—and reaction progress was tracked by real-time heat-flow reaction calorimetry (Fig. 2) (24). The contrast in rate was drastic: The catalyzed cycloaddition rapidly reached completion, producing copper triazolide **4** within 20 min (tables S2 and S3), whereas the noncatalyzed reaction showed no appreciable conversion to the triazolide (table S4). Lowering the catalyst concentration resulted in a corresponding decrease in the maximum rate, showing a positive-order dependence on the exogenous copper species (fig. S3). Moreover, multiple soluble exogenous copper sources exhibited similar catalytic efficacy (fig. S4), and organic azides bearing sterically encumbered or aliphatic functional groups showed comparable rate acceleration in the presence of an added copper catalyst (fig. S5 and table S5) (25). Similarly, a marked difference in the rates of the catalyzed and noncatalyzed reactions initially shown in THF was observed in other solvents, chloroform and dimethylformamide (25) (fig. S6).

With the necessity for a second copper atom within the active cycloaddition complex established, we sought to determine the respective roles

of each copper atom. Given the well-documented stability of the copper-carbene complexes (26) and the robust nature of acetylide **2**, we hypothesized that the two copper atoms act in discrete and specialized roles, with copper in acetylide **2** acting purely as a strongly σ -bound ligand, whereas the second copper atom operates exclusively through weak π -complexation (Fig. 1C, where $\text{X} = \text{Cu}$). To probe this hypothesis, we prepared an isotopically pure ^{63}Cu exogenous catalyst to differentiate it from the naturally abundant distribution of copper isotopes (^{63}Cu : ^{65}Cu ratio of

69:31) contained in the preformed copper(I)-acetylide. The copper(I) coordination complex $[\text{Cu}(\text{MeCN})_4]\text{PF}_6$ **5** (Me, methyl) (27), which is an air-stable, crystalline solid, was selected for the stoichiometric crossover studies and synthesized in short order from the isotopically pure, commercially available copper(II) oxide (99.9% ^{63}Cu).

As both the acetylide reactant **2** and the copper triazolide product **4** contain a copper atom, we designed a stoichiometric crossover experiment in which the absence of isotopic

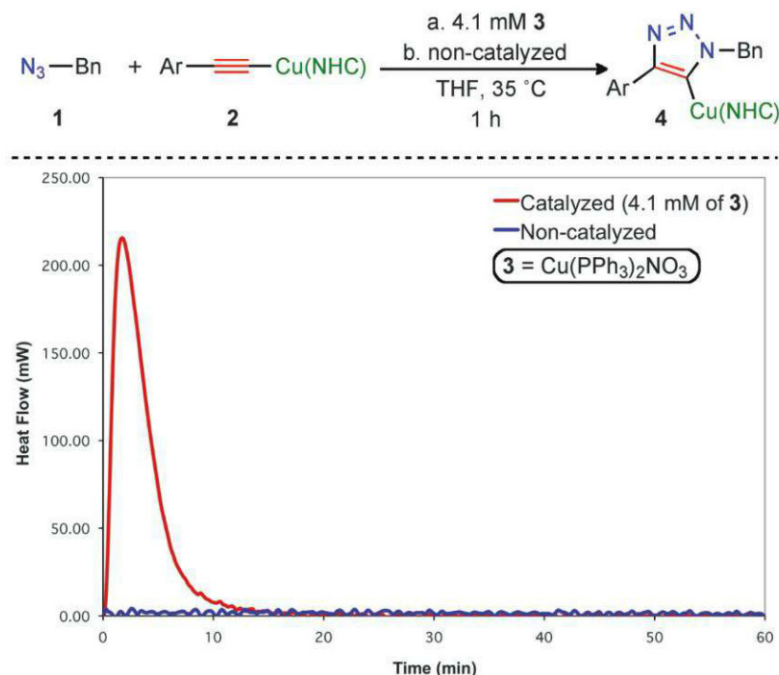
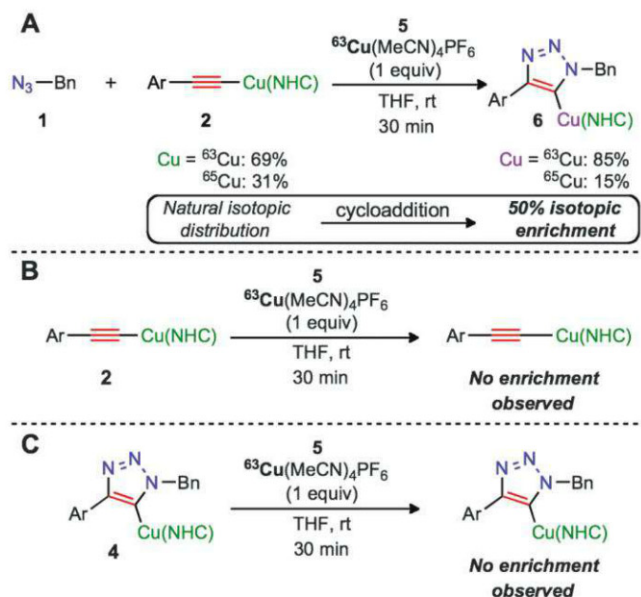


Fig. 2. Reaction progress. Global heat (mW, milliwatts) output graph for the catalyzed and uncatalyzed formation of triazolide **4**. Bn, benzyl; Ar, *p*-^tBuPh; ^tBu, *tert*-butyl; NHC, 1,3-bis(2,6-diisopropyl)phenyl-4,5-dihydroimidazol-2-ylidene.

Fig. 3. Isotope crossover studies. (A) Observed isotopic enrichment of triazolide **6**. (B) No isotopic enrichment of acetylide **2** observed. (C) No isotopic enrichment of triazolide **4** observed. rt, room temperature.



enrichment of the metal center of the resulting triazolide would support the hypothesized independent roles of the two copper species. Thus, we combined azide **1**, acetylide **2** (natural isotopic ratio of $^{63/65}\text{Cu}$), and isotopically enriched copper complex **5**, and azide **1** (in analogy to the initial crossover experiment) and analyzed the reaction mixture by TOF-MS. Again, no enrichment of acetylide **2** in the presence of azide **1** was noted (figs. S11 and S12). Thus, although organic azides could act as ligands for the copper(I) complex, evidently this interaction is not sufficient to mediate the observed enrichment event.

With acetylide **2** excluded as a possible intermediate for exchange, we next examined whether the isotopic enrichment occurred after the formation of triazolide **4**. Accordingly, we combined azide **1**, acetylide **2**, and a catalytic amount of $[\text{Cu}(\text{MeCN})_4]\text{PF}_6$ (natural isotopic distribution, 5 mole percent) in THF to fully form the nonenriched triazolide **4**. We added a stoichiometric equivalent of the enriched copper complex **5** to this solution (and tracked the results by TOF-MS), from which no enrichment of triazolide **4** was observed (Fig. 3C and fig. S13), excluding it as a venue for the observed enrichment.

By process of elimination, the observed 50% isotopic enrichment must occur within the cycloaddition steps (boxed intermediates in Fig. 4A). We propose the following mechanistic interpretation to account for these results (Fig. 4A): First, the σ -bound copper acetylide bearing the π -bound enriched copper atom **7** reversibly

coordinates an organic azide, forming complex **8**. Following this step, nucleophilic attack at N-3 of the azide by the β -carbon of the acetylide forms the first covalent C–N bond, producing intermediate **9**. The ligand exchange in this intermediate (isomers shown in Fig. 4B) is faster than the formation of the second covalent C–N bond, which results in ring closure, accounting for the statistical (50%) incorporation of ^{63}Cu into triazolide **6**. The exclusive formation of triazolide **6** indicates a thermodynamic preference for the NHC-bound copper triazolide; however, rapid ligand exchange renders equal the probability for either copper atom (natural or enriched) to be incorporated in the resulting intermediate. We attribute the unprecedented lability and rapid exchange of the NHC ligand between the two copper atoms in intermediate **9** to the weakened copper-carbene backbonding (29–31), as the formal oxidation state of copper increases upon the formation of the first C–N bond. The degenerate, proximal copper centers in **9** facilitate this oxidation event, with the second copper atom acting as a stabilizing donor ligand to the otherwise highly energetic and unstable mononuclear metallacycle intermediate (**13**) (Fig. 1B). Taken together with prior studies of the full catalytic cycle, these experiments support the mechanistic model for the CuAAC, featuring two chemically equivalent copper atoms working in concert for the regioselective formation of 1,4-substituted 1,2,3-triazoles (Fig. 4C and fig. S14).

The in situ reaction calorimetry and metal isotope crossover methods used in this study

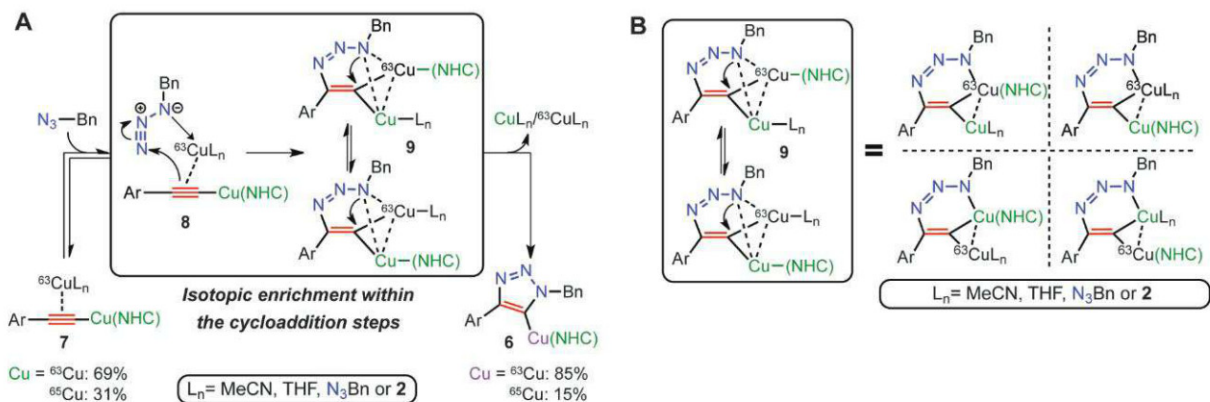


Fig. 4. Mechanistic conclusions. (A) Mechanistic rationale for the isotopic enrichment of triazolide **6**. (B) Rapidly interconverting isomers of intermediate **9**. (C) Proposed catalytic model for the CuAAC with two copper atoms.

have permitted us to deduce the involvement of unstable, non-isolable intermediates that have previously eluded more conventional mechanistic studies. Similarly innovative studies that delineate reactivity patterns of more capricious but abundant metals (Cu, Fe, Ni) are needed to further understand and develop new catalytic processes, reducing synthetic reliance on well-behaved but rare and expensive transition metals (Pd, Rh, Ru). Moreover, the mechanistic insights presented in this study, specifically the formation of the active cycloaddition complex with two differentiable copper atoms, imply a unified reactivity of electronically rich σ -acetylides (1-halo- or 1-metallo-) with 1,3-dipoles (e.g., azides, nitrile oxides, and nitrones). As such, we propose a common reactivity trend in which any σ -acetylide that can effectively recruit a π -bound copper atom will undergo annulation with a compatible dipolar partner.

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Supplementary Materials

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Supplementary Text

Figs. S1 to S14

Tables S1 to S5

NMR Spectra

References (32–34)

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Observations of Ejecta Clouds Produced by Impacts onto Saturn's Rings

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We report observations of dusty clouds in Saturn's rings, which we interpret as resulting from impacts onto the rings that occurred between 1 and 50 hours before the clouds were observed. The largest of these clouds was observed twice; its brightness and cant angle evolved in a manner consistent with this hypothesis. Several arguments suggest that these clouds cannot be due to the primary impact of one solid meteoroid onto the rings, but rather are due to the impact of a compact stream of Saturn-orbiting material derived from previous breakup of a meteoroid. The responsible interplanetary meteoroids were initially between 1 centimeter and several meters in size, and their influx rate is consistent with the sparse prior knowledge of smaller meteoroids in the outer solar system.

Quantifying the mass flux onto Saturn's rings has been a long-desired goal. Mass infall likely dominates the rings' coloration and their levels of non-ice pollution, as well as influencing angular momentum transport and erosion of particles, all of which in turn have the potential to set limits on the age of the rings (1–3). Impacts may be a trigger mechanism for the “spokes” observed in the B ring (4, 5), and they may be responsible for producing some dust outbursts observed in the F ring (6, 7). Additionally, direct measurement of the mass infall

onto Saturn's rings would constrain the population of interplanetary meteoroids in the outer solar system, which is poorly determined for macroscopic particles (1, 8). However, although impact flashes have been observed on Earth's Moon [e.g., (9)], efforts to detect impact flashes in the outer solar system have been unsuccessful (10, 11).

Here, we report images of ejecta clouds above Saturn's rings (Fig. 1), obtained by Cassini's Imaging Science Subsystem (12, 13). These features are normally not visible owing to low contrast

with the background ring, but there are two particular viewing geometries in which they become visible: (i) during saturnian equinox, at which time the background ring becomes very dark, and (ii) when Cassini views the rings at high resolution and very high phase angle, at which time the dusty features appear very bright (Fig. 2).

The saturnian equinox, which occurs every ~14.5 years and is closely accompanied by edge-on viewing of Saturn's rings from Earth, has led to many scientific discoveries over the centuries since 1612, when the ring system's disappearance from Galileo's view gave the first hint of its disk geometry (14). The 2009 equinox was the first for which an observer (namely, the Cassini spacecraft) was in place at close range and high saturnian latitudes. During a few days surrounding the precise moment of the Sun's passage through the ring plane, the main rings provided an unusually dark background, due to the edge-on illumination, while anything (e.g., an ejecta cloud) extending vertically out of the ring plane

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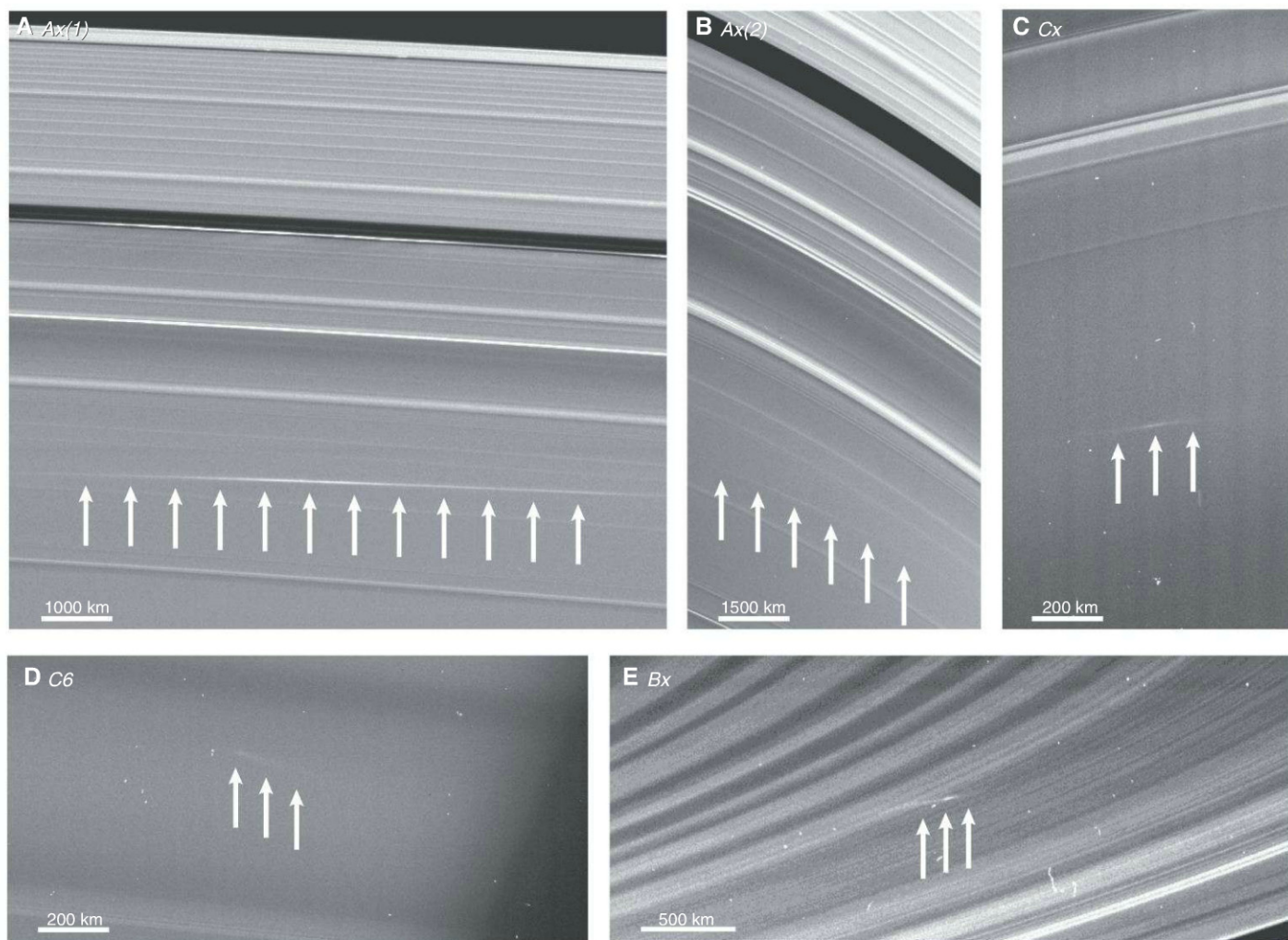
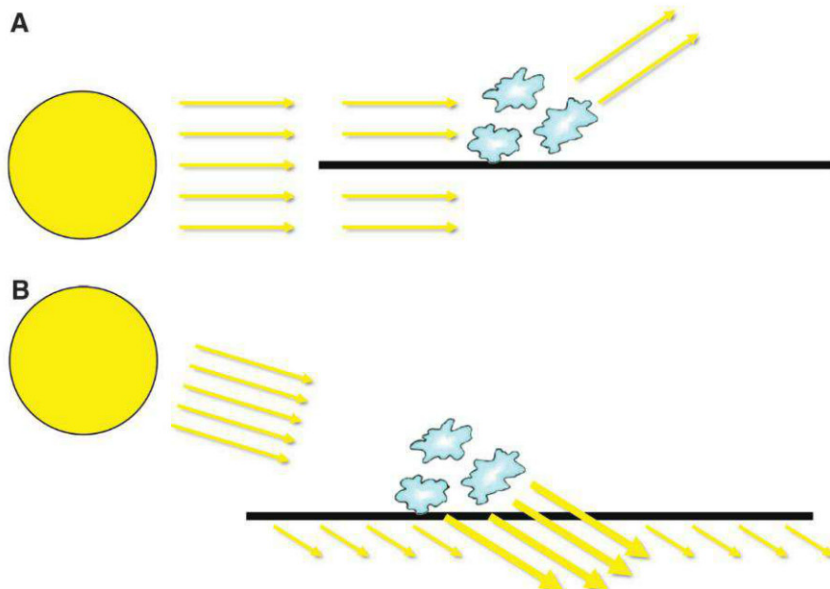


Fig. 1. Ejecta clouds. (A and B) Two views of one cloud in the A ring, taken 24.5 hours apart [code-named Ax(1) and Ax(2)]; (C) a cloud in the C ring (code-named Cx); (D) another cloud in the C ring (code-named C6); and (E) a cloud in the B ring (code named Bx). Arrows point to the cloud structures, which are visibly canted from the surrounding azimuthally aligned features. The images shown here were all taken during the 2009 saturnian

equinox event except (D) (containing C6), which was taken at high resolution and very high phase angle in 2012. The clouds are reprojected onto a radius-longitude grid in Fig. 3A and fig. S2. The features C1 through C5, which were seen in the C ring in 2005 at geometries similar to that for C6 and which are smeared owing to orbital motion of the features during the image exposure, are shown in figs. S1 and S3.

Fig. 2. Viewing geometry makes the clouds visible. (A) In this cartoon, sunlight (yellow) approaches the rings (black) nearly edge-on during the equinox event, causing them to appear very dark. However, a cloud of ejecta above the rings (blue) can scatter light toward the camera. (B) In this cartoon, sunlight is scattered forward (i.e., at high phase angles) much more efficiently by the dusty ejecta cloud than by the largely dust-free main ring.



was fully illuminated (Fig. 2A). The observed bright streaks (code-named “Ax,” “Bx,” and “Cx,” after the ring region in which each feature was seen, with “x” indicating that it was seen during the equinox event) are measured to be canted (Fig. 3, A and B) by a few degrees from the azimuthal direction. The feature Ax, observed on two occasions, showed a cant angle θ decreasing with time. The most likely reason for an ejecta cloud to evolve in this way is if it was produced quickly (for example, within a fraction of an hour, much less than the orbital periods of between 6 and 14 hours in the rings), with subsequent evolution of each constituent particle on an independent Keplerian orbit. An impact is the most likely way to produce an ejecta cloud so quickly.

We also report here observations of smaller ejecta clouds at nonequinox times in highly forward-scattering geometries (i.e., at phase angle $\alpha > 172^\circ$), in which dust (i.e., micrometer-size) particles brighten considerably as a result of diffraction. Free-floating dust is not abundant in the background ring, as it gets swept into the regoliths of larger particles (15), so the dusty ejecta clouds appeared with greatly enhanced contrast in this geometry (Fig. 2B). Cassini images at such high phase angles are only possible when the spacecraft is passing through the planet’s shadow, as the safety of its optical instruments would be jeopardized by pointing them so close to the Sun’s direction at any other time; furthermore, the small features we report here can only be seen when Cassini is at close range to the rings. These conditions were met for four images in 2005, in which we report five observed ejecta clouds (code-named “C1” through “C5”; fig. S1) that are smeared owing to orbital motion of the features during the image exposure. Furthermore, a sixth ejecta cloud (“C6”; Fig. 1E) was seen in follow-up images in 2012.

The most interesting feature in our data set is Ax, of which two independent observations allow us to investigate the evolution of both its cant angle and its brightness.

Particles with initially identical longitudes on circular orbits with different radial distances will drift apart in longitude because of Keplerian shear, a straightforward consequence of Kepler’s third law. Thus, the shape of an initially compact cloud will quickly become dominated by a cant angle θ (Fig. 3B and fig. S5), which is given by (supplementary text 3)

$$\tan \theta = \frac{P}{3\pi t} \quad (1)$$

where t is the time elapsed and P is the orbital period. This curve is plotted as the solid line in Fig. 3C, where it is seen that the measured cant angles for apparitions Ax(1) and Ax(2) make an excellent fit to the model, implying that Ax was 1.8 orbital periods old (i.e., 23.5 hours) when it was first observed.

We considered the possibility that the observed ejecta clouds were created by the impact of a single solid object with the rings. Because

such an impact would throw ejecta in every direction from a single impact point, the cant angle $\theta(t)$ would evolve in a more complex manner due to the noncircularity of ejecta orbits, and we found that this model makes a much poorer fit to the observations (supplementary text 3). The solid impactor model also cannot readily account for the horizontally aligned initial structure of Ax (supplementary text 5) and the volume of observed ejecta (supplementary text 9), so we will assume henceforth that the impacting mass was a broader (but still well-confined) stream of impacting material.

The measured brightness evolution of Ax also supports its identification as an ejecta cloud. Ejecta raised above an orbiting planetary ring does not “land” in the same way as in the more familiar scenario of ejecta raised above a solid surface. Rather, because of Kepler’s laws of orbital motion, the ejecta simply pass through the ring once every half-orbit at low relative speeds, and only a fraction equal to $e^{-\tau}$ (where τ is the ring’s optical depth) will encounter ring material and be removed from the cloud. The time

elapsed between the two observations of Ax corresponds to four half-orbits, regardless of the impactor model discussed above. Thus, we can use the ratio of the two observations of the cloud’s total integrated brightness to independently calculate the optical depth of Saturn’s A ring (supplementary text 4). Accounting for the different geometries of the two observations, we obtain $\tau \sim 0.65$ for the A ring, in good agreement with other independent measurements of this quantity (16) and confirming our interpretation of the observed structures as evolving ejecta clouds.

Several of the observed ejecta clouds, including Ax(1), Bx, and C6, are asymmetric, such that the brightest point is noticeably offset from the apparent center of the cloud (Fig. 1). When the effects of the cant angle are removed, the core structure of Ax(1) is revealed to be azimuthally elongated by a factor of ~ 7 (fig. S8). We interpret this to constrain the length and width of the impactor stream (supplementary text 5), which we suggest was formed from an interplanetary meteoroid that broke apart as a result of an impact and entered saturnian orbit upon its

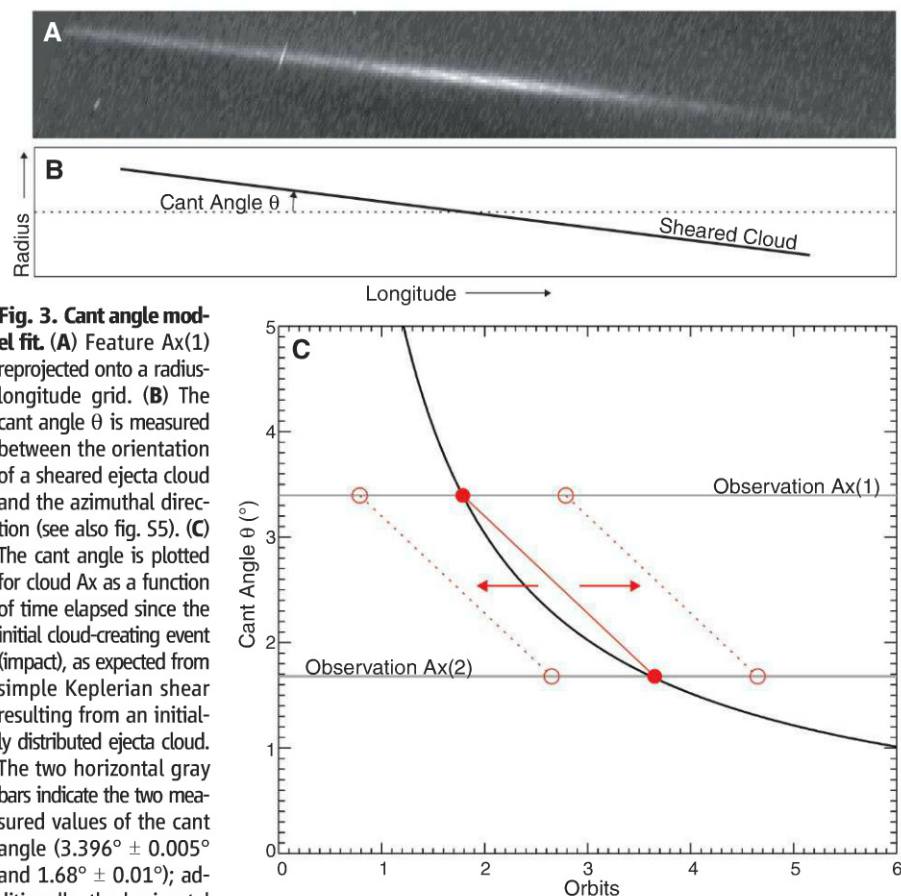


Fig. 3. Cant angle model fit. (A) Feature Ax(1) reprojected onto a radius-longitude grid. (B) The cant angle θ is measured between the orientation of a sheared ejecta cloud and the azimuthal direction (see also fig. S5). (C) The cant angle is plotted for cloud Ax as a function of time elapsed since the initial cloud-creating event (impact), as expected from simple Keplerian shear resulting from an initially distributed ejecta cloud. The two horizontal gray bars indicate the two measured values of the cant angle ($3.396^\circ \pm 0.005^\circ$ and $1.68^\circ \pm 0.01^\circ$); additionally, the horizontal distance between each pair of red circles is fixed by the time elapsed between the two measurements (24.5 hours, or 1.86 orbital periods), so the line connecting each pair has a fixed length and orientation. However, the assemblage can slide horizontally because the time of the initial impact is not directly known (the directions in which the solution can vary are indicated by the red arrows, open circles, and dotted lines). The solid red circles connected by the solid red line indicate where the solid curve passes through the first cant angle measurement, resulting in a residual at the second cant angle measurement of only 0.02° , which is 0.9σ given our estimated measurement error (supplementary text 3).

first encounter with the rings [compare to (17)], then produced the observed ejecta cloud upon its second encounter. Keplerian shear would cause the stream to spread along-track to the inferred length in ~ 8 hours, implying that the impacting material reached an apoapse of $\sim 160,000$ km before returning to the ring (supplementary text 5).

The peripheral regions of feature Cx (Fig. 1 and fig. S9) have a steeper cant angle than the bright central region, possibly because the different parts of the cloud have different ages, though it may be that the central region is somehow distended and that the cant angles in the peripheral regions give the feature's true age (supplementary text 6).

Feature C6 was imaged at multiple wavelengths. Its reddish slope (18) could be explained by a deficit of particles in the ejecta cloud with radii smaller than $s_{\min} \approx 3 \mu\text{m}$ (supplementary text 7).

From our measurements of the total integrated brightness of each observed feature (supplementary text 2), the ejecta mass can be estimated (supplementary text 8) if we make an assumption

about the particle-size distribution $n(s)$ of the ejecta. For simplicity, we use a power-law model $n(s) = n_0 s^{-q}$, where $n(s)ds$ is the number of particles with radius between s and $s + ds$, though the real particle-size distribution may be more complex. Under this model, we find that the cloud mass is a strong function of q . In particular, when we assume minimum and maximum particle sizes $s_{\min} = 3 \mu\text{m}$ and $s_{\max} = 3 \text{ cm}$ (supplementary text 8), the cloud mass is four orders of magnitude larger for $q < 3$ than it is for $q > 4$ and transitions quickly between the two values for $3 < q < 4$ (fig. S10).

As discussed above, we can estimate the initial ejecta mass by multiplying the mass inferred for the observed cloud by a factor $e^{N\tau}$ (supplementary text 8), where N is the number of half-orbits completed since the cloud formed as inferred from its cant angle, and τ is the optical depth of the ring at the relevant location (19). In the absence of detailed calculations of impacts into rings, we then estimate the mass of the impactor by assuming that the ejecta mass is greater

than the impacting mass by a yield factor $Y \approx 10^4$ (supplementary text 9). Using the dimensions of each observed feature to estimate the area of the ring affected by impacting material, we find that the available target ring mass is sufficient to account for the observed cloud only if q is not too small and/or $s_{\max}$ is not too large (or, equivalently and more generally for the case of non-power-law particle-size distributions, if large ejecta particles are not too abundant). We estimate the Ax impactor to have been between 1 and 10 m in radius before breakup, with the uncertainty mostly due to the particle-size distribution, the assumed yield, and other systematic effects (fig. S11). For the same reason, we estimate the other ejecta clouds reported here to have been due to impactors with radii between 1 cm and 1 m before breakup.

Using the fractional area coverage of the respective ring for each sequence of images that detected a feature described in this work, and taking each observed ejecta cloud's apparent age (table S2) as an estimate of the time for which it remained visible, we estimate the influx of meteoroids to the Saturn system (Fig. 4 and supplementary text 10). This empirical estimate of centimeter-to-meter-size meteoroids in the outer solar system fills a gap between measurements of micrometer-size dust by Pioneer, Ulysses, and New Horizons (1, 8, 20) and ~ 10 -km objects that are seen directly. To date, little is known about small macroscopic particles in the outer solar system, with most investigators assuming that the Pioneer and Ulysses data justify the extrapolation of measurements in Earth's vicinity.

Our results are higher than the extrapolation by one or two orders of magnitude (Fig. 4); at face value, especially if this increase is also reflected in submillimeter-to-millimeter-size particles, then pollution and erosion rates due to interplanetary meteoroids might be higher than have been thought. However, gravitational focusing enhances the flux at Saturn's rings by a factor of 4 to 40 (20, 21), and the sensitivity of rings to a two-directional flux yields another factor of 2, so our results may corroborate the previous extrapolation after all.

No ejecta clouds were observed in the region dominated by spokes, though we cannot rule out the possibility that spoke-forming impacts may also form ejecta clouds. The intersection between our size distribution and the observed spoke-forming rate (21, 22) indicates that spokes are due to meteoroids of radius ~ 10 to 50 cm (supplementary text 11).

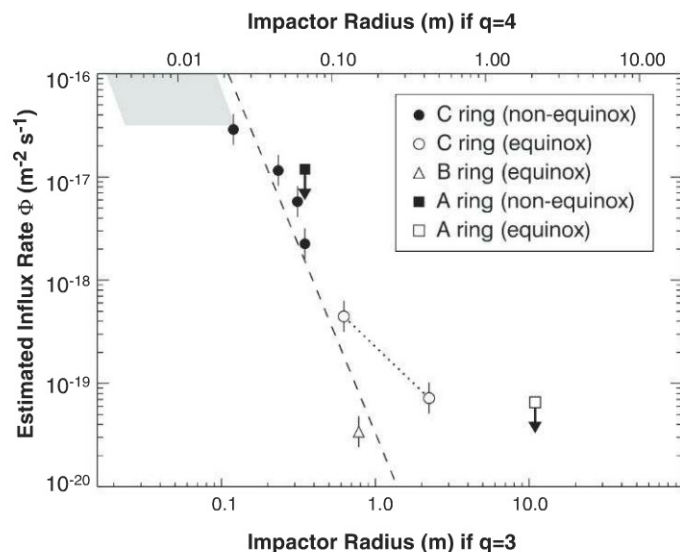


Fig. 4. Impactor size spectrum. Estimated cumulative influx rate of interplanetary meteoroids at 10 astronomical units (AU), not corrected for Saturn's gravitational focusing, as a function of inferred impactor radius (before preliminary breakup). The vertical error bars indicate the Poisson uncertainty in estimating the influx rate from a small number of events. Horizontal uncertainties in the individual data points (e.g., from the measurement of the cloud's brightness) are smaller than the plotted symbols, but systematic uncertainties that may cause all the data points to move horizontally together are quite large. The largest systematic uncertainty is the particle-size distribution (represented by the power-law index q) of the observed clouds, which is indicated by the difference between the upper and lower horizontal axes; other sources of systematic error include the yield factor Y , the albedo ω_0 , and the phase function $P(\alpha)$ (supplementary texts 2 and 9), which may further move the horizontal axis by as much as a factor of several. The gray region is the micrometeoroid population estimated by scaling the near-Earth population by outer solar system dust measurements (1, 8); its horizontal breadth is the systematic uncertainty due to q . As detailed in supplementary text 10 and figs. S6 and S7, the lowest filled circle is the 2012 detection of C6, whereas the other three filled circles are the 2005 detections of C1 through C5. The filled square with downward-pointing arrow is the nondetection of A-ring objects in the sequence of images that captured C6. The open triangle is equinox feature Bx. The two open circles connected by a dotted line represent the peripheral regions (left) and central region (right) of equinox feature Cx; the peripheral regions (the left-hand circle) may be the more reliable data point (supplementary texts 6 and 9). The open square is Ax, with downward-pointing arrow to indicate that the detection may be coincidental rather than indicative of the influx rate (supplementary text 10). The dashed line is fitted to all the data points that lack arrows. The fitted cumulative number flux of interplanetary meteoroids at 10 AU is $\Phi \approx (3 \times 10^{-9} \text{ m}^{-2} \text{ s}^{-1}) R_{\text{impactor}}^{-3.6}$ for $q = 3$ and R_{impactor} in meters.

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Supplementary Materials

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Supplementary Text
Figs. S1 to S11
Tables S1 to S7
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Melting of Iron at Earth's Inner Core Boundary Based on Fast X-ray Diffraction

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Earth's core is structured in a solid inner core, mainly composed of iron, and a liquid outer core. The temperature at the inner core boundary is expected to be close to the melting point of iron at 330 gigapascal (GPa). Despite intensive experimental and theoretical efforts, there is little consensus on the melting behavior of iron at these extreme pressures and temperatures. We present static laser-heated diamond anvil cell experiments up to 200 GPa using synchrotron-based fast x-ray diffraction as a primary melting diagnostic. When extrapolating to higher pressures, we conclude that the melting temperature of iron at the inner core boundary is 6230 ± 500 kelvin. This estimation favors a high heat flux at the core-mantle boundary with a possible partial melting of the mantle.

Earth's inner core grows by solidification from the surrounding outer core, which is composed of molten iron (Fe) alloyed

with ~10 weight percent light elements (1). Seismological data reveal important physical properties of the core, such as density (and, hence,

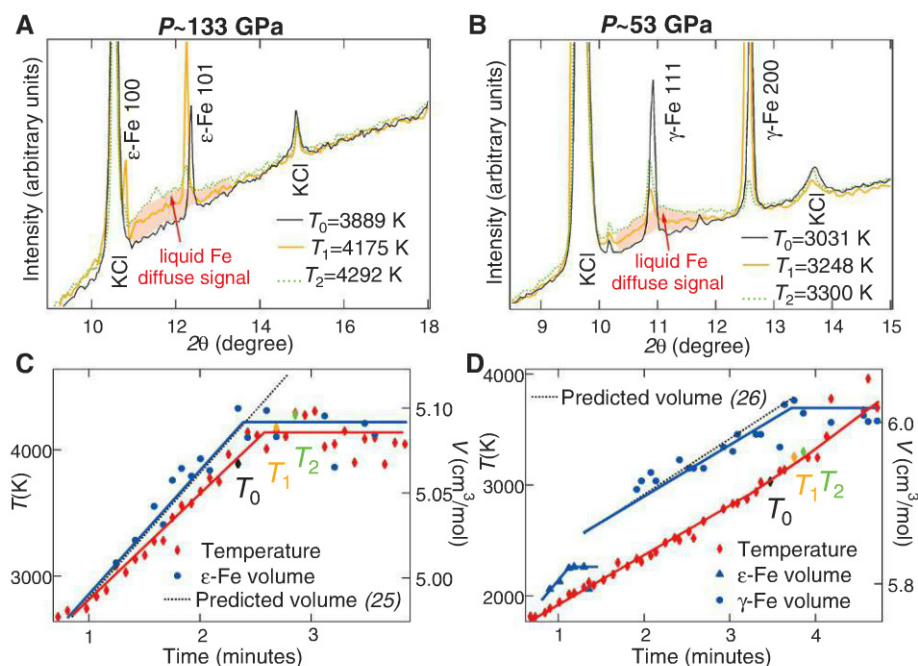
pressure) and elasticity; however, they cannot directly reveal the corresponding temperature. Temperature in the core places important constraints on parameters like heat flux to the mantle, power for the geodynamo, and cooling rate, all of which are fundamental to Earth's heat budget and dynamics (2).

The temperature at the inner core boundary (ICB) is bracketed between the melting temperature of pure Fe at 330 GPa and the liquidus temperature of the outer core iron-rich alloy (3) [expected to be depressed by ~700 K (4)]. Neither dynamic (5–7) and static (8–11) compression measurements nor thermodynamic modeling (12–15) have resulted in a consensus on ICB melt-

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Fig. 1. XRD patterns as a function of temperature, pressure, and time. (A and B) XRD patterns of a ~3-μm-thick (~7-μm-thick) sample during a heating series at $P \approx 133$ GPa and 53 GPa, respectively, recorded at different temperatures. γ -Fe, ϵ -Fe, and KCl pressure medium diffraction peaks are labeled. Liquid Fe is evidenced by a diffuse ring at $2\theta = 10^\circ$ to 13.5° . (C and D) Pyrometry temperature, measured and predicted (25, 26). Fe volume as a function of time for the thin (C) and thick (D) samples. The laser's power is linear with time. Data are in table S1.



ing temperature, in part because these approaches suffer from intrinsic uncertainties. Dynamic measurements have long been considered the most promising way to determine the ICB temperature because shocked Fe melts around 230 GPa, yet the temperature determination (spanning from

5100 to 6350 K) and the possibility of superheating in shock compression (16) are major uncertainties. Using laser heating in static diamond anvil cell (DAC) experiments to produce reliable melting data above 100 GPa is also difficult because of uncertainties in the pyrometric

temperature measurements (17), the criterion used to identify the melting (18), and the possible temperature-induced chemical reactions (18, 19). In fact, estimation of Fe melting temperature at ICB pressure based on static compression data spans the range 4850 (8) to 7600 K (10). Finally, the tremendous advances in computational capacity have enabled quantum-mechanics calculations of the melting behavior, but each method has underlying approximations or assumptions. For instance, melting temperatures from 6370 (14) to 7050 K (12) have been obtained with the same melting criterion—the coexistence of a liquid and solid phase in a molecular dynamics run—but a different description of interatomic forces within density functional theory. Going beyond density functional theory with a quantum Monte Carlo simulation, melting was obtained at 6900 K (15) at 330 GPa.

Here, we report the laser-heated DAC determination of the Fe melting curve from 50 to 200 GPa, using a μm -spatial and second-time-resolved approach that has recently been applied to the determination of the Ta melting line (18). Fast x-ray diffraction (XRD) is used as the primary technique for structural determination. This approach presents several advantages: (i) The structural evolution of Fe can be followed during heating; (ii) the measured volume expansion of solid Fe provides an independent control of temperature measurements; (iii) chemical reactions, if any, can be observed within the few percentage detection limits; and (iv) most importantly, an unambiguous bulk signature of melting—i.e., the appearance of a diffuse ring—is recorded (9, 20). This technique offers an alternative to the melting diagnostics used in the past in the laser-heated DAC: motion of the sample surface (8, 10), microscopic observation of the recovered samples (10), and plateaus/drops on the temperature ramps (8, 21).

The XRD spectra obtained during heating provide direct information about the physical state of the laser-heated sample that can be correlated with additional information such as temperature T versus time (Fig. 1). From this, we determined that $\epsilon\text{-Fe}$ (hexagonal close packed) and $\gamma\text{-Fe}$ (face-centered cubic) are the only structures observed in the investigated pressure-temperature range. This confirms earlier findings of a large stability field for $\epsilon\text{-Fe}$ (19, 22) and extends in temperature this domain up to the melting line at 200 GPa—e.g., under the conditions where a transformation to a body-centered cubic phase had been suggested using shock wave measurements (23) or ab initio calculations (24). XRD patterns also show, in a few cases, a partial reaction of Fe with the diamond anvil, as shown by the appearance of weak peaks that can be assigned to Fe_3C (20). Therefore, each heating series was performed on a fresh, unheated zone of the sample. Furthermore, we measured and compared the volume of solid Fe with the expected volume based on the pyrometry temperature and the equation of state of $\epsilon\text{-Fe}$ (25) or $\gamma\text{-Fe}$

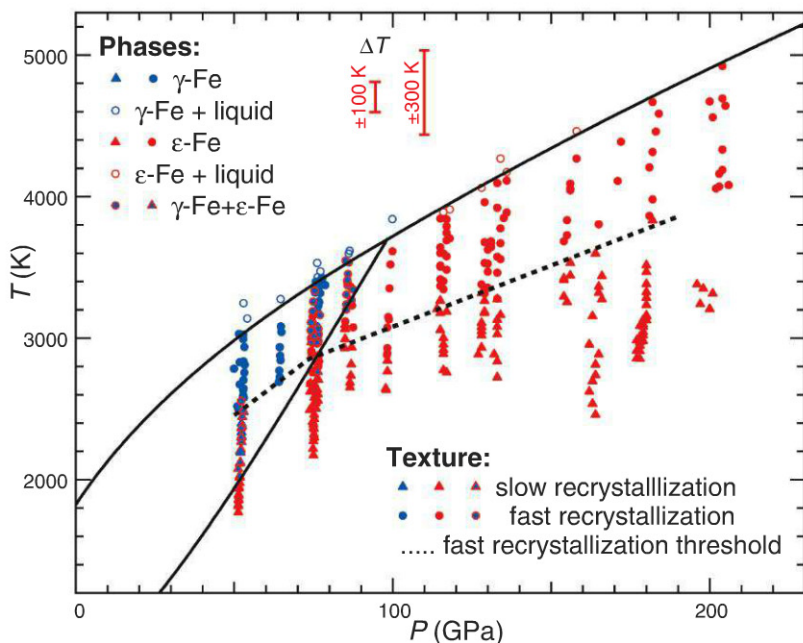


Fig. 2. Pressure (P_{KCl})-temperature conditions at which XRD patterns have been collected. Different symbols correspond to different Fe phases and textures. The continuous black lines correspond to Eqs. 1, 2, and 3. Data are in table S1.

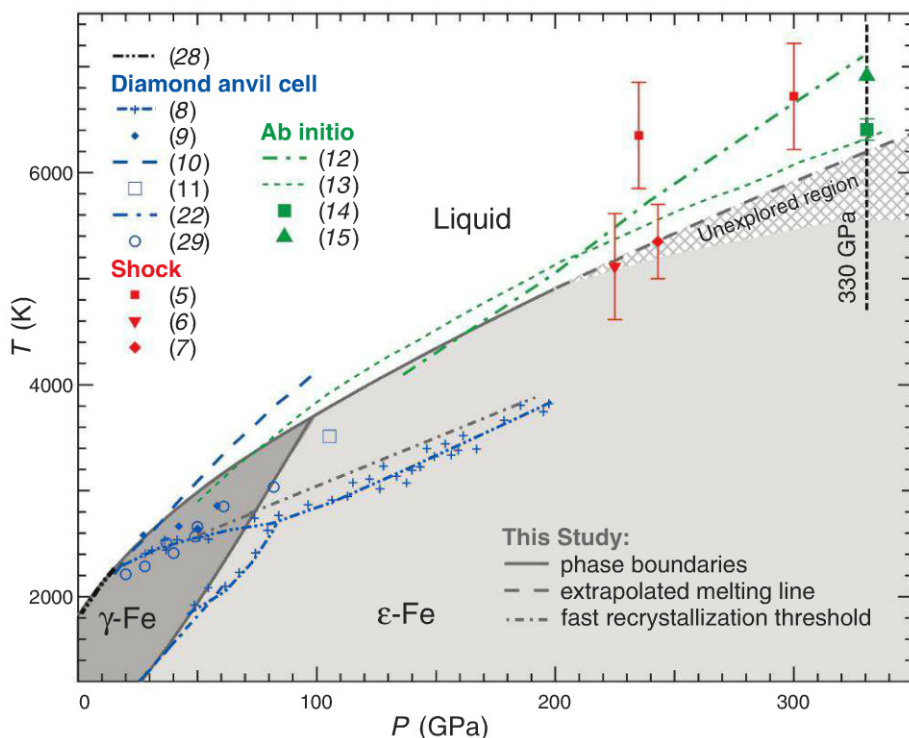


Fig. 3. Phase stability domains for Fe obtained in the literature and in this study. The stability field for $\epsilon\text{-Fe}$ is based on the current study data and data from (19).

(26), assuming that the pressure remained constant. In cases where an inconsistency was detected, the ramp data was discarded. Finally, XRD images yield information on the sample texture: The starting fine powder transformed into large single crystals upon heating. Above a certain temperature, single-crystal spots appeared and disappeared at each XRD exposure, which we refer to as “fast recrystallization.”

Changes in T versus time curves, such as plateaus or sudden drops, are often considered as a melting signature (8, 21). This is interpreted as a loss of thermal insulation in the sample assembly due to melting (27). We observed such features, but not systematically. In Fig. 1, the temporal evolutions of the temperature and the ϵ -Fe volume of a $\approx 3\text{-}\mu\text{m}$ thickness sample are parallel: a linear increase, then a marked plateau. A diffuse scattering signal of the liquid appears in the XRD signal at the beginning of this plateau; however, ϵ -Fe single crystal XRD spots indicate that the sample is not completely molten. For a thicker ($\approx 7\text{ }\mu\text{m}$) sample, the observations seem less correlated. After the expected γ -Fe to ϵ -Fe phase transition (22), the ϵ -Fe volume has a similar behavior as for the thin sample: It plateaus during melting, evidenced by the diffuse XRD signal. However, the pyrometry temperature continues to increase. With the rise of temperature, the intensity of the liquid signals increases, indicating that the amount of molten Fe scanned by XRD increases. Our interpretation is that the solid Fe is surrounded by layer of liquid Fe a few μm thick, whose surface is heated and analyzed by pyrometry. We observe that this layer sustains a sharp temperature gradient, its surface being up to 400 K warmer than solid Fe. The XRD measurement of the plateau of thermal expansion and the simultaneous observation of the diffuse diffraction peak of the liquid provide here an unambiguous signature of melting.

The melting temperature T_m is estimated as the average between, respectively, the highest (lowest) temperatures at which only solid (solid + liquid) Fe is observed (Fig. 2). The temperature of fast recrystallization is substantially (400 to 900 K) lower than the melting temperature. Two Simon equations are used to fit the melting points together with existing low-pressure data (28). The ϵ -Fe to γ -Fe phase boundary is estimated using the current data and literature data (22, 28). The γ - ϵ -liquid triple point is at a pressure of 98.5 GPa (P_{TP}) and 3712 K (T_{TP}). The following formulations are obtained [T in K, P in GPa, $T_0 = 1991$ K, and $P_0 = 5.2$ GPa (28)].

$$T = 575 + 18.7P + 0.213P^2 - 0.000817P^3$$

$$(\epsilon - \text{Fe}/\gamma - \text{Fe}) \quad (1)$$

$$(P - P_0)/27.39 = (T_m/T_0)^{2.38} - 1$$

$$(\gamma - \text{Fe}/\text{liquid}) \quad (2)$$

$$(P - P_{TP})/161.2 = (T_m/T_{TP})^{1.72} - 1$$

$$(\epsilon - \text{Fe}/\text{liquid}) \quad (3)$$

The present melting curve closely follows the one determined by Alfè *et al.* (13) on the basis

of a Gibbs free-energy minimization (Fig. 3). It goes through the dynamic determinations of melting (6, 7), around 230 GPa. The melting temperatures of Fe obtained with the laser-heated DAC are bracketed by two studies (8, 10), which mainly differ in their melting criteria. The present melting curve is within the error bars of the measurements based on the observation of textural changes on recovered samples up to 100 GPa (10)—a method which is probably not possible above that pressure—and agrees correctly with determinations based on a similar method (9, 11) and a recent indirect measurement (29). The current melting points are up to 1000 K higher than the melting points obtained by the observation of surface motion of a laser-heated sample (8). Neither temperature measurement techniques, which are similar, nor pressure calibration issues can explain such a difference. It is possible that the melting diagnostic used in (8) detects fast recrystallization instead of melting. Indeed, it is interesting to note that the melting line of (8) coincides with the temperature of fast recrystallization, observed in this study and in another recent work (30). In the latter study, it was interpreted as melting evidence. We propose instead that fast recrystallization begins at temperatures below melting and causes the surface motion observed in (8), which had been wrongly attributed to melting.

Eq. 3 extrapolates to $T_m(330\text{ GPa}) = 6230 \pm 500$ K. The error is the sum of the maximum pyrometry error and the uncertainty on the detection of melting temperature (200 K). A temperature profile inside Earth's core can be estimated using this value, which leads to a temperature at the core-mantle boundary (T_{CMB}) of 4050 ± 500 K (20). This corresponds to a thermal boundary layer of ~ 1400 K at the base of the mantle and, subsequently, a high CMB heat flux (~ 10 TW) (2). Such a value is needed to sustain the geodynamo with recent estimate of the core conductivity (31). At CMB pressure, partial melting of the mantle material has been observed at 4180 ± 150 K (32) and would therefore be possible with strong geodynamical and geochemical implications. At 135 GPa, the post-perovskite phase is stable below 3520 ± 70 K for MgSiO_3 (33); a double-crossing scenario (33) is therefore compatible with the current T_{CMB} , given the error of experimental determinations.

Our experiments result in better agreement between calculations, and dynamic and static DAC approaches, and reduce uncertainty on the expected melting temperature of Fe at the ICB pressure. Because this study spans the 50 to 205 GPa range, we cannot rule out the possibility of a phase transition in Fe at higher P - T (unexplored region in Fig. 3), which could slightly change the extrapolation of the current determination of the Fe melting curve.

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Supporting Materials

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Early Ceremonial Constructions at Ceibal, Guatemala, and the Origins of Lowland Maya Civilization

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The spread of plaza-pyramid complexes across southern Mesoamerica during the early Middle Preclassic period (1000 to 700 BCE) provides critical information regarding the origins of lowland Maya civilization and the role of the Gulf Coast Olmec. Recent excavations at the Maya site of Ceibal, Guatemala, documented the growth of a formal ceremonial space into a plaza-pyramid complex that predated comparable buildings at other lowland Maya sites and major occupations at the Olmec center of La Venta. The development of lowland Maya civilization did not result from one-directional influence from La Venta, but from interregional interactions, involving groups in the southwestern Maya lowlands, Chiapas, the Pacific Coast, and the southern Gulf Coast.

During the early Middle Preclassic period (1000 to 700 BCE), sedentary communities using ceramics began to appear in the Maya lowlands (Figs. 1 and 2). Many of their neighbors were already leading sedentary ways of life and were using ceramics during the Early Preclassic period (before 1000 BCE) (*1*). In particular, the inhabitants of southern Veracruz and western Tabasco, generally called the Gulf Coast Olmec, were building a large center at San Lorenzo and were producing elaborate stone sculptures (*2, 3*). Scholars have long debated the influence that the Gulf Coast Olmec had on the development of lowland Maya society. Some view the Gulf Coast Olmec as the mother culture: the source of cultural innovations from which characteristic art styles and centralized political organization spread to the Maya and other Mesoamerican groups (*4–6*). Others contend that the lowland Maya received only limited Olmec influence or interacted with them as competing peers (*7–9*). A critical issue is the development of formal architectural complexes consisting of plazas and pyramids, which eventually became the hallmarks of Mesoamerican civilizations. San Lorenzo did not have substantial mounds or pyramids (*2, 10*). The subsequent Gulf Coast center of La Venta, in contrast, exhibited a highly formalized arrangement of a pyramid, platforms, and plazas, and it has been suggested that this architectural template developed there spread to the Maya lowlands and other parts of southern Mesoamerica (*5, 11*). Here we describe excavations at the Maya center of Ceibal that provide evidence of earlier architecture and thus imply a more complex process.

Ceibal (also spelled Seibal) is located in the southwestern part of the Maya lowlands and was explored extensively in the 1960s by a team

from Harvard University (*12*). These studies showed that its occupation began during the early Middle Preclassic period characterized by Real ceramics (*13*). We targeted group A, where the Harvard team found concentrations of Real ceramics (Fig. 3). Our excavations revealed early Middle Preclassic buildings in four areas: the core of structure A-20 and the plaza in front of structure A-20, as well as in the lower levels of the A-24 platform and the East Court. Structure A-20 appears to have at least 11 construction

stages, of which 5 or more date to the Real phase (Fig. 4). The excavation results and radiocarbon dates indicate that around 1000 BCE, the early residents of Ceibal made the first version of structure A-20 (Ajaw) by carving a high point of the limestone bedrock and placing black soil with high clay content on top (fig. S1). This building measured 2.0 m in height and 4.0 m in the east-west dimension of its summit. Thus, the first version of A-20 was a low flat platform rather than a pyramid. On the eastern side of the building, a ramp carved out of natural marl (decaying limestone bedrock) provided access to the summit. A major renovation took place during the Real 2 phase (850 to 800 BCE), and the expanded building (B'ehom) appears to have reached a height of 3 to 5 m and had a pyramidal shape. We estimate that the building measured roughly 6 to 8 m in height by the end of the Real phase around 700 BCE. Fifty meters to the east of structure Ajaw, we uncovered a long platform (Xa'an) buried under later plaza fills. Structure Xa'an was also carved out of natural marl around 1000 BCE, and it measured 1.0 m in height and probably 42 to 55 m in length. Structures Ajaw and Xa'an represent the earliest known example of the so-called "E-Group assemblage," a ceremonial compound consisting of a western square structure and an eastern long platform that became ritual foci of many lowland



Fig. 1. Map of southern Mesoamerica with the locations of Ceibal and the sites discussed in the text.

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Maya centers during later periods. At the same time, the residents scraped off the surface soil in the area between structures Ajaw and Xa'an to create a leveled plaza and used the exposed whitish marl as the first plaza floor. Numerous offerings, including greenstone axes, were deposited in this plaza, indicating that this space served as the primary stage of communal ritual throughout the Middle Preclassic period. During the Real 3 phase (800 to 700 BCE), the Ceibal residents expanded this plaza by burying structure Xa'an in a plaza fill and constructing a new version of the eastern long platform (Saqupsin) farther to the east.

The earliest version of the A-24 platform (Sulul), revealed in operation (Op.) 200B, was also built around 1000 BCE (Fig. 5 and fig. S2). It consisted of sticky dark clay that appears to have been taken from the wetlands and was placed directly over the irregular natural ground surface. This fill measured 1.3 m in thickness, but the platform's horizontal dimensions are unknown. Platform Sulul was soon expanded horizontally to reach an east-west dimension of more than 34 m, as indicated by the find of the same wetland-soil fill in Op. 200A. The total thickness of platform Sulul's fill in Op. 200A measured more than 2.5 m because of the sloping natural terrain, suggesting that the construction of this platform involved a substantial investment of labor. During the latter part of the Real 1 phase, the Ceibal residents expanded this platform vertically using clay mixed with silt and sand. Through at least 17 episodes of construc-

tion by the end of the Real 2 phase, the renovated version of the platform (Ch'och') reached total fill thicknesses of 3.5 m in Op. 200B and roughly 6 m in Op. 200A. During the Real 3 phase, the A-24 platform received little renovation, and the focus of construction activity shifted to the northeastern platform, the East Court. This platform started with one or two episodes of substantial construction with earthen fills (K'at), possibly during the Real 2 phase, which measured 1.4 to 1.7 m in total thickness. By the end of the Real phase, platform K'at had received at least 15 episodes of reflooring and rebuilding, reaching a fill thickness of 2.0 to 2.3 m. In contrast to structures Ajaw, Xa'an, and Saqupsin of a public and ceremonial nature, platforms Sulul/Ch'och' and K'at appear to have supported multiple residential structures, which consisted of low clay or clay-and-stone foundations and perishable walls and roofs. Excavations of the East Court revealed parts of three structures dating to the late Middle Preclassic Escoba phase, which appear to have surrounded a square patio. We suspect that a residential complex in a similar configuration existed during the Real phase.

We obtained 54 radiocarbon dates, to which we applied Bayesian modeling to develop a site chronology (figs. S3 and S4 and table S1). Multiple dates associated with the earliest versions of structures Ajaw and Xa'an and platform Sulul concentrate around 1000 BCE, marking the beginning of the Real 1 phase. The following Real 2 phase is dated to 850 to 800 BCE with 13 overlapping radiocarbon dates. This facet is more

precisely dated because the radiocarbon calibration curve of this span is steep, and the radiocarbon dates obtained from a sequence of numerous floors in platform Ch'och' made Bayesian modeling effective. The date of the Real 3 phase is less precise because of the flattening of the calibration curve, but we estimate that it spanned from 800 to 700 BCE.

The beginning date of 1000 BCE for Ceibal corresponds roughly with the onset of sedentary settlements with ceramics in other parts of the Maya lowlands, including Cuello, Cahal Pech, Blackman Eddy, and Tikal (14–17). Although some of these sites have yielded earlier calibrated dates, acceptable dates from secure contexts tend to fall around 1000 BCE or later (18). Furthermore, these sites share ceramics with post-slip incisions characteristic of the early Middle Preclassic period. However, formal ceremonial complexes dating to 1000 to 800 BCE have not been found at these other sites; excavations have revealed only humble residences, consisting of postholes dug in the natural ground or small platforms less than half a meter in height. The Ceibal E-Group assemblage consisting of structures Ajaw and Xa'an thus predates other known examples from the Maya lowlands by two centuries or more. Komchen may have substantial platforms dating to the early Middle Preclassic period, but their initial configuration is not clear, and their dates remain imprecise. During the Real 2 phase, the Ceibal E-Group assemblage grew to be the earliest known plaza-pyramid complex in the Maya lowlands, and structure

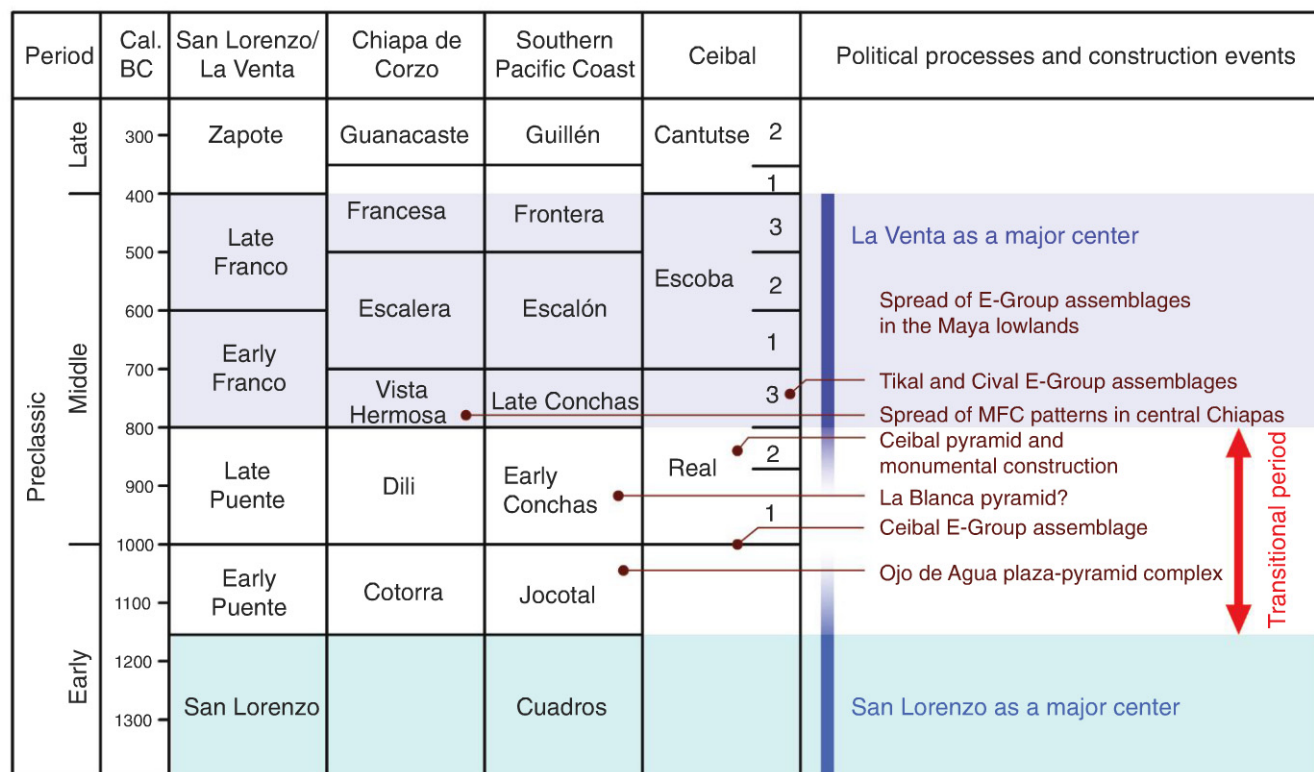


Fig. 2. Chronology of southern Mesoamerica.

B'ehom and platform Ch'och' represented the earliest monumental constructions. The next oldest E-Group assemblages in the Maya low-

lands have been found at Tikal and Cival (7, 17). They are associated with ceramics contemporaneous with the Real 3 and Escoba 1 phases (800

to 600 BCE). During the late Middle Preclassic period (700 to 400 BCE), E-Group assemblages with pyramids spread to numerous lowland Maya centers, including Nakbe.

Outside the Maya lowlands, interpretations hinge on a comparison with La Venta. Much of our knowledge of this Olmec center comes from excavations in the 1940s and 1950s, and a large part of the site is unexplored. Its chronology has been a vexing problem. New radiocarbon dates and the cross-dating of ceramics and figurines, however, suggest to some that the rise of La Venta as a major center occurred after 800 BCE (19–21). We applied Bayesian statistics to these radiocarbon dates, using the improved regional ceramic sequence (22, 23). Out of 10 significant cultural features excavated in complexes E and G near the La Venta ceremonial core, 9 belong to the Franco ceramic phase (800 to 400 BCE), characterized by composite-silhouette bowls and plates (round bases and walls that are straight or curve out), black serving vessels, and hatched triangles and other incised or grooved motifs (figs. S5 and S6 and table S2). These results support the interpretation that the population of La Venta grew rapidly after 800 BCE. The construction of La Venta's ceremonial core may have started earlier (fig. S7 and table S3), but before 800 BCE La Venta was probably a smaller, less influential center and may have had mutually stimulating interactions with Ceibal and other contemporaneous communities. These dates also indicate that there was a substantial gap between the decline of San Lorenzo and the growth of its successor. The interpretation of San Lorenzo's fall has been equally uncertain, although the date of 1000 BCE has traditionally been used (2). However, a Bayesian model of available dates suggests that San Lorenzo's heyday more likely ended around 1150 BCE (figs. S8 and S9 and table S4). Moreover, archaeological data from central Chiapas and the Chiapas Pacific Coast, as well as the Bayesian statistics of radiocarbon dates from there (figs. S10 and S11 and table S5), indicate that the influence of San Lorenzo diminished around this date (the end of the Cuadros phase on the Pacific

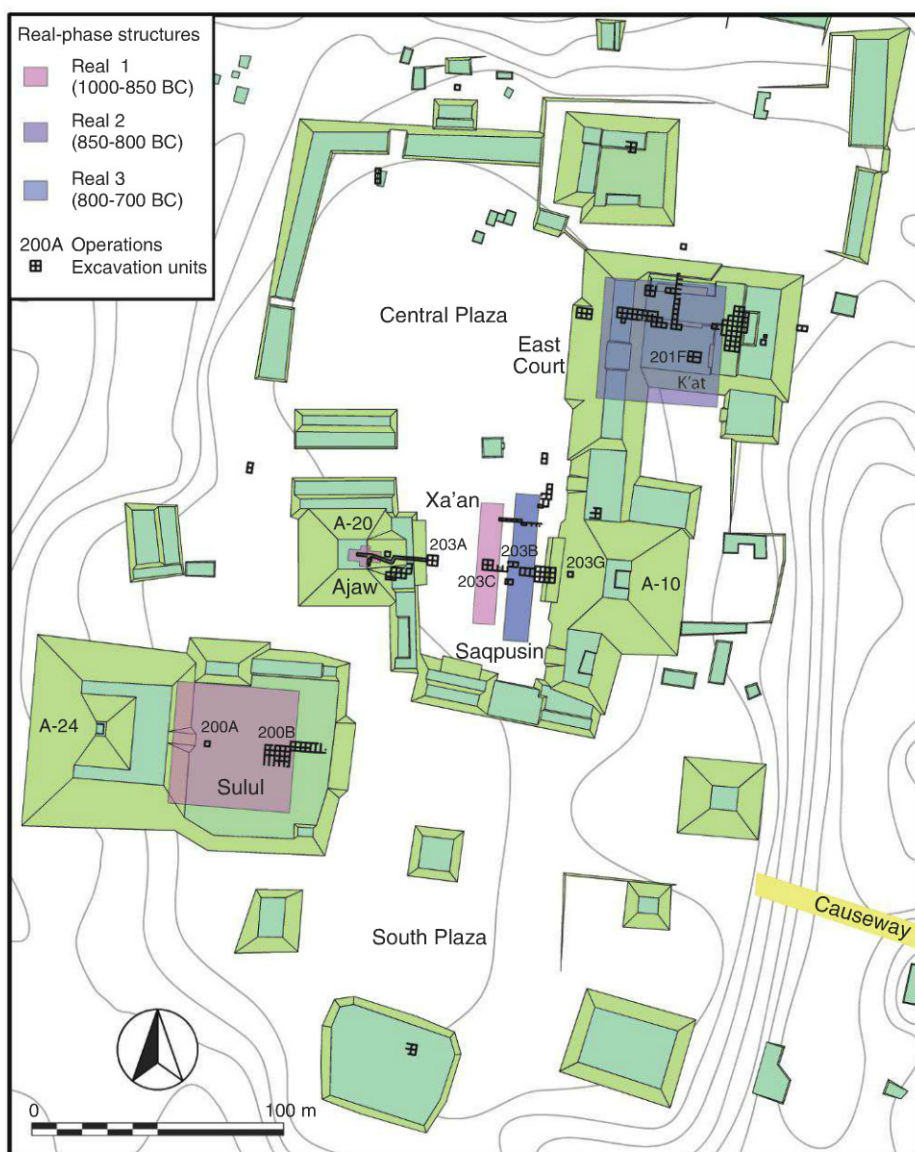


Fig. 3. Map of Ceibal group A with the locations of excavation units and Real-phase structures.

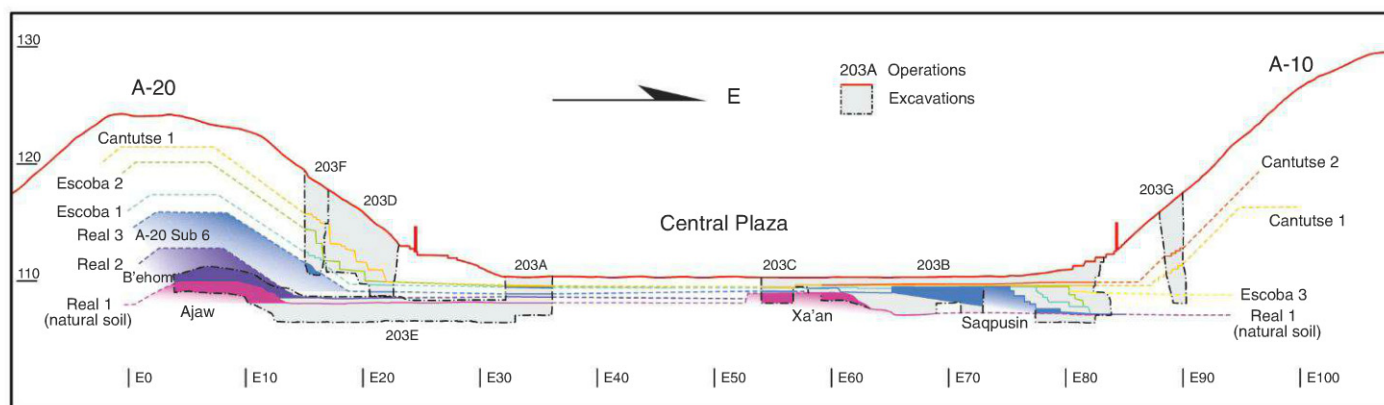


Fig. 4. East-west section of the central plaza and structures A-10 and A-20.

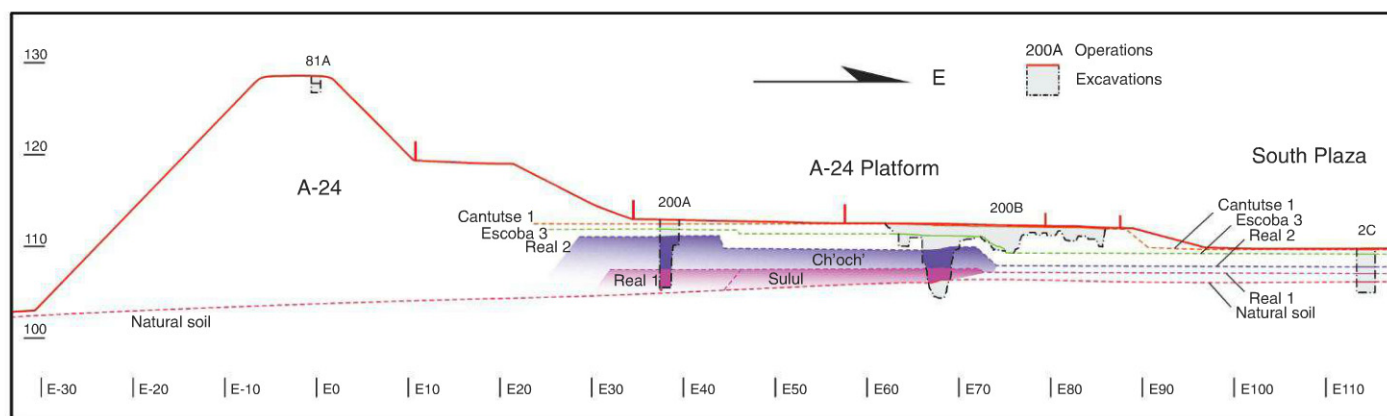


Fig. 5. East-west section of the A-24 platform.

Coast and that of the Cacahuanó phase in the western Grijalva River basin) (19, 24, 25). The establishment of formal ceremonial spaces and their growth into plaza-pyramid complexes probably occurred during this transitional period between the two Gulf Coast powers.

An important area for the development of ceremonial complexes was probably central Chiapas along the Grijalva River. Clark, following McDonald and Lowe (5, 11, 26), noted that Middle Preclassic centers in this region, including Mirador, San Isidro, Ocozocoautla, Chiapa de Corzo, Finca Acapulco, and La Libertad, as well as Tzutzuculi on the Pacific Coast, shared highly standardized spatial plans, each consisting of an E-Group assemblage and large platforms placed along the north-south axis of the settlements. This configuration, which Clark called the Middle Formative Chiapas (MFC) pattern, closely resembles that of Ceibal. Many of the MFC-pattern complexes, including those at Tzutzuculi, Mirador, San Isidro, and La Libertad, were not constructed until 800 or 700 BCE (26–29). The E-Group assemblage at Chiapa de Corzo may be slightly earlier (30). Excavations also suggest that, at the beginning of the Middle Preclassic, the central part of Chiapa de Corzo was leveled off, and scraped surface soils along with remains of the Early Preclassic period were dumped to the slope near the ceremonial core (31). This means that the early residents of Chiapa de Corzo and Ceibal shared similar manners of preparing the areas at the onset of ceremonial constructions, apparently emphasizing a break from the previous era. The excavation results of Finca Acapulco have not been fully described, but it is possible that this site with significant Early Preclassic occupations had ceremonial constructions contemporaneous with those of Ceibal (25). An even earlier plaza-pyramid complex may have existed on the Chiapas Pacific Coast. During the Jocotal phase (1200 to 1000 BCE), when the influence of San Lorenzo weakened, a series of extensive mounds were constructed near estuaries, but the most impressive was Ojo de Agua in an inland area (32). Its pyramids, substantial platforms, and plazas exhibit a vague resemblance

to the MFC pattern. The southern Pacific Coast had a tradition of building residences on raised platforms associated with plazas dating back at least to the Locona phase (1600 to 1500 BCE) (33). However, during the Cuadros phase (1350 to 1200 BCE), when influence from San Lorenzo was notable, the largest community of the area, Cantón Corralito, adopted San Lorenzo-like constructions lacking tall mounds but consisting of an extensive low terrace that supported residential buildings with low foundations (24). This sequence suggests that the plaza-pyramid complex of Ojo de Agua did not derive from the Gulf Coast. During the following Conchas phase (1000 to 600 BCE), the center of La Blanca across the Guatemalan border grew, with a large pyramid, mound 1, eventually reaching a height of 25 m. The date of La Blanca mound 1 remains problematic (34). Its monumental construction may predate those at Ceibal and La Venta or may be contemporaneous with them.

It is well documented in North and South America that monumental architecture could be built long before the emergence of centralized politics and marked social inequality (35). Critical issues in this study then are not only the sizes of buildings but also the social roles that buildings and spaces played in specific historical contexts. The data from Ceibal make it clear that the original emphasis was on the ceremonial complex as a stage for communal ritual performances. Pyramids resulted from a sequence of architectural renovations applied to original platforms and possibly provided spatial settings for more marked segregations among community members (36). The emergence of standardized ceremonial complexes exemplified by the E-Group assemblage and the MFC pattern were possibly associated with increasingly prescribed forms of interactions and shared notions of new social order (37). The sequential architectural development at Ceibal implies that this center was not a passive recipient of an idea established elsewhere, but it most likely participated actively in the process of this innovation. This development appears to have occurred through interregional interactions, primarily involving groups in the

southwestern Maya lowlands, Chiapas, the Pacific Coast, and the southern Gulf Coast. Then around 800 to 400 BCE, plaza-pyramid complexes spread to other parts of southern Mesoamerica, including the central Maya lowlands, the Guatemalan highlands, El Salvador, and Honduras. The residents of highland Mexico, including the Basin of Mexico, Morelos, and Oaxaca, built substantial platforms during the Early Preclassic period, but they were slow to adopt pyramidal architecture (38–40). These observations indicate that the origins of lowland Maya civilization can be explained neither in terms of Gulf Coast Olmec influence nor independent development. One-directional influence from La Venta was probably not significant at the beginning of the Middle Preclassic period, but interactions with and inspirations from the area to the west were still critical for social changes in the Maya lowlands.

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Supplementary Materials

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PINK1-Phosphorylated Mitofusin 2 Is a Parkin Receptor for Culling Damaged Mitochondria

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Senescent and damaged mitochondria undergo selective mitophagic elimination through mechanisms requiring two Parkinson's disease factors, the mitochondrial kinase PINK1 (PTEN-induced putative kinase protein 1; PTEN is phosphatase and tensin homolog) and the cytosolic ubiquitin ligase Parkin. The nature of the PINK-Parkin interaction and the identity of key factors directing Parkin to damaged mitochondria are unknown. We show that the mitochondrial outer membrane guanosine triphosphatase mitofusin (Mfn) 2 mediates Parkin recruitment to damaged mitochondria. Parkin bound to Mfn2 in a PINK1-dependent manner; PINK1 phosphorylated Mfn2 and promoted its Parkin-mediated ubiquitination. Ablation of Mfn2 in mouse cardiac myocytes prevented depolarization-induced translocation of Parkin to the mitochondria and suppressed mitophagy. Accumulation of morphologically and functionally abnormal mitochondria induced respiratory dysfunction in Mfn2-deficient mouse embryonic fibroblasts and cardiomyocytes and in Parkin-deficient *Drosophila* heart tubes, causing dilated cardiomyopathy. Thus, Mfn2 functions as a mitochondrial receptor for Parkin and is required for quality control of cardiac mitochondria.

Mitochondria are endosymbiotic organelles derived from primitive aerobic bacteria. Healthy mitochondria are essential energy generators for most metazoan

cell functions, whereas senescent or damaged mitochondria are sources of toxic reactive oxygen species. Thus, mitochondrial biogenesis and mitophagic elimination are carefully orchestrated, and their mutational disruption causes chronic degenerative diseases (1). Genetic studies have linked Parkinson's disease to mutations of two mitophagy genes, the E3 ubiquitin ligase Parkin and the serine-threonine protein kinase

PINK1 (PTEN-induced putative kinase protein 1; PTEN is phosphatase and tensin homolog) (2). Loss of the mitochondrial inner membrane electrochemical gradient stabilizes PINK1 on damaged organelles, tagging them for Parkin binding, ubiquitination, and mitophagic elimination (3). The specific nature of the molecular interaction between PINK1 and Parkin is unclear, and Parkin receptor proteins on damaged mitochondria have not been identified.

Mitofusins (Mfn) 1 and 2 are mitochondrial outer-membrane fusion proteins and Parkin ubiquitination substrates (4, 5). Combined genetic ablation of Mfn1 and Mfn2 in mouse hearts induces mitochondrial dysfunction and fragmentation that should stimulate mitophagic removal but instead results in proliferation of abnormal organelles (6). Because mitophagy is stimulated by Parkin-mediated ubiquitination of mitochondrial proteins (7, 8), we tested whether Mfn1 or Mfn2 might mediate signaling activity of the PINK1-Parkin pathway. Parkin co-immunoprecipitated with Mfn2, but not Mfn1, from extracts of human embryonic kidney (HEK) cells transfected with tagged mitofusins and Parkin. The association of Mfn2 and Parkin was greatly enhanced in cells also transfected to overexpress PINK1 (Fig. 1, A and B, and fig. S1).

The oxidative phosphorylation inhibitor carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) depolarizes mitochondria and stimulates PINK1-mediated translocation of Parkin to mitochondria, thus targeting damaged organelles for mitophagy (3) (fig. S2). Consistent with a role

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for Mfn2 in this process, FCCP treatment stimulated Parkin binding to endogenous fibroblast Mfn2 (fig. S3). Overexpression of PINK1 enhanced, whereas PINK1 suppression with RNA interference inhibited, this effect of FCCP (fig. S3). We developed an *ex vivo* model of this pathway in cardiomyocytes because they express endogenous Mfn2 and Parkin in large amounts and have abundant mitochondria. In genetically normal cardiomyocytes, mitochondrial uncoupling with FCCP (fig. S4) directed redistribution of Parkin to intracellular punctae especially profuse in the mitochondrial-rich perinuclear region (Fig. 1C, left). Parkin translocation was stimulated by FCCP in Mfn1-null, but not Mfn2-null, cardiomyocytes (Fig. 1C, middle and right). In Mfn2-null cells, Parkin remained diffusely cytosolic, demonstrating a requirement for endogenous Mfn2 to promote Parkin localization to depolarized mitochondria. Because Mfn1 and Mfn2 are each expressed in normal amounts in hearts lacking their opposite number (fig. S5) and recombinant Mfn1 does not bind Parkin (Fig. 1A), Parkin recruitment appears to be a unique property of Mfn2.

Mfn2 can be a Parkin ubiquitination substrate (8). This might require PINK1-stimulated asso-

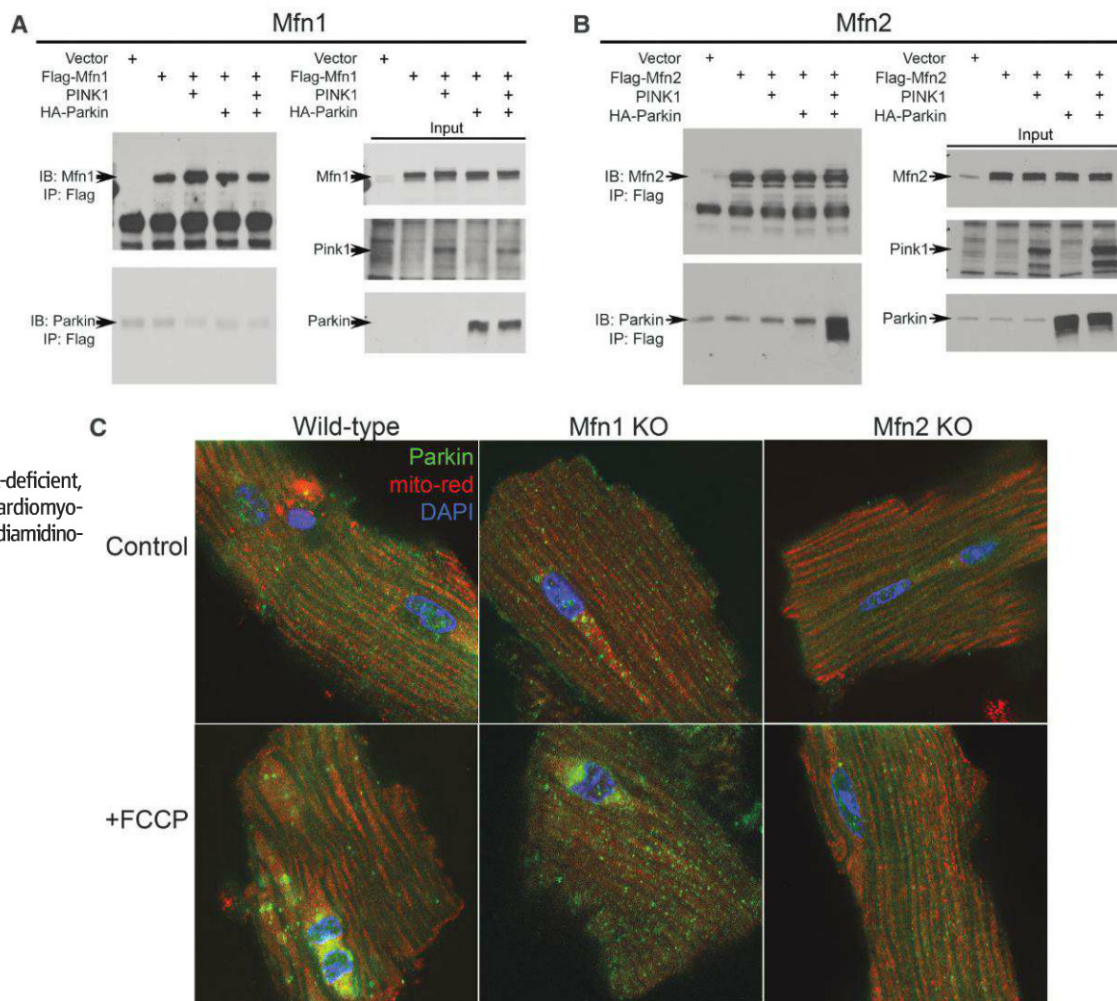
ciation of Parkin and Mfn2 with mitochondria, so we tested the functional importance of PINK1-Mfn2-Parkin interactions on ubiquitination. We observed complete concordance of ubiquitination with PINK1-stimulated Mfn2 binding to Parkin in HEK cells (Fig. 2A) and with FCCP-stimulated binding of endogenous cardiac Mfn2 to Parkin in isolated perfused mouse hearts (Fig. 2B). Conversely, Mfn2 (but not Mfn1) gene ablation in cardiac myocytes decreased mitochondrial ubiquitination stimulated by mitochondrial depolarization, following the same pattern seen for Parkin translocation (Fig. 2C and fig. S6). Moreover, the mitophagic response, measured as punctal accumulation of the mitophagy adaptor protein p62 [also called sequestosome 1 (SQSTM1)], was impaired in FCCP-treated Mfn2-deficient cardiac myocytes (Fig. 2D and fig. S6). Together, these data support a model in which Mfn2 functions as a receptor to which cytosolic Parkin binds on depolarized mitochondria, provoking ubiquitination of mitochondrial proteins that target the organelle for autophagic elimination.

Enhancement of Mfn2-Parkin association by PINK1 indicated that Mfn2 might be phosphorylated. We noted a slight electrophoretic mobility

shift in a fraction of Mfn2 when PINK1 was co-transfected, suggesting a possible posttranslational modification (Fig. 3A, top). This mobility shift was augmented on Phos-tag (Wako Chemicals USA, Incorporated, Richmond, Virginia) gels, indicating that Mfn2 might be phosphorylated at one or more sites (Fig. 3A, bottom, and fig. S7). Indeed, phosphoserine immunoreactivity of immunoprecipitated Mfn2 was also increased in cells overexpressing PINK1 (Fig. 3B). If PINK1-mediated phosphorylation of Mfn2 is required for Mfn2-Parkin interactions, then catalytically inactive PINK1 should be ineffective. Consistent with this notion, the catalytically inactive PINK1 Lys²¹⁹, Asp³⁶², Asp³⁸⁴ → Ala mutant (9) failed to promote Mfn2-Parkin binding or to induce the characteristic change in Mfn2 mobility on Phos-tag gels (Fig. 3C).

To establish that Mfn2 is a substrate of PINK1 and the mitochondrial binding partner for Parkin, we mapped the Mfn2 PINK1 phosphorylation sites and examined their functional consequences. Bioinformatics analysis identified three highly conserved potential Mfn2 phosphorylation sites: Thr¹¹¹ (T111), Ser⁴⁴² (S442), and Tyr⁴⁴⁸ (Y448) (figs. S8 and S9). Because PINK1 is a serine-threonine kinase, we mutated Mfn2 T111 and

Fig. 1. Interaction of Mfn2 with Parkin in a PINK1-dependent manner and its requirement for Parkin translocation to depolarized mitochondria. (A and B) Fibroblasts were transfected with Flag-Mfn1 (A) or Mfn2 (B), PINK1, and/or hemagglutinin (HA)-Parkin; immunoprecipitated (IP) with anti-Flag; and immunoblotted (IB). Right-hand gels show IB of input homogenates. **(C)** Subcellular Parkin redistribution (green) induced by mitochondrial depolarization with FCCP in wild-type, Mfn1-deficient, and Mfn2-deficient mouse cardiomyocytes. KO, knockout; DAPI, 4',6-diamidino-2-phenylindole.



S442 to alanine, preventing their phosphorylation. Both Mfn2 mutations decreased PINK1-stimulated Mfn2-Parkin binding without eliminating it (Fig. 3D), whereas simultaneous mutation of both residues (T111A/S442A) completely abrogated PINK1-stimulated interactions between Mfn2 and Parkin (Fig. 3E). In agreement with these loss-of-function data, Mfn2 mutations that mimic PINK1 phosphorylation of Mfn2 (T111E/S442E) conferred PINK1-independent binding activity to Parkin (Fig. 3E). Collectively, these studies reveal a potential mechanism by which Mfn2 orchestrates the PINK1-Parkin mitochondrial quality control apparatus.

We tested whether decreased Parkin translocation caused by the absence of its mitochondrial binding partner would adversely affect long-term mitochondrial homeostasis. Because it has no Parkin binding activity, deletion of Mfn1 had no effect on mitochondrial morphometry or respiratory function of cardiomyocytes (fig. S10, A and B). However, deletion of Mfn2 provoked mitochondrial enlargement, which we measured by ultrastructural examination (Fig. 4A and fig. S10A) and flow-cytometric forward light scatter (fig.

S10C). Mfn2-null cardiac myocytes also exhibited decreased substrate-dependent O_2 consumption, pointing to mitochondrial respiratory impairment (Fig. 4B).

Mitochondrial function deteriorates over time in senescent hearts, contributing to increased prevalence of heart failure with age (10). Because mitochondrial abnormalities caused by Mfn2 ablation resemble those of age-related cardiomyopathy, we assessed *in vivo* cardiac chamber dilation and contractile function over time in hearts from cardiac-specific Mfn1 and Mfn2 knock-out mice. Hearts lacking Mfn1 appeared normal (fig. S11A) and had normal heart function assessed through noninvasive echocardiography (fig. S11B) or invasive hemodynamic measurements of contractility ($+dP/dt$, the maximal rate of increase in left ventricular pressure over time) (fig. S11C). However, hearts lacking Mfn2 dilated with increasing age (fig. S11D), developed impaired contractile performance assessed by echocardiography (Fig. 4C and fig. S11E), and were insensitive to β -adrenergic stimulation (fig. S11F). If cardiomyopathy in hearts lacking Mfn2 is caused by the defect in Parkin signaling, then

primary Parkin deficiency should recapitulate the organelle and organ phenotypes. Indeed, in *Drosophila* lacking Parkin (11) cardiomyocyte mitochondria were enlarged (Fig. 4D), and heart tubes exhibited impaired respiration (Fig. 4E) with chamber dilation and contractile impairment (Fig. 4F and fig. S12). Parkin-deficient cardiomyocyte mitochondria were not effectively ubiquitinated after FCCP stimulation (Fig. 4G). The common properties of Mfn2-deficient mouse hearts and Parkin-deficient *Drosophila* heart tubes are characteristic of maladaptive remodeling leading to dilated cardiomyopathy. The inability of Parkin to promote ubiquitin-dependent mitophagy in both models may contribute to accumulation of abnormal mitochondria that ultimately impairs cellular respiration and compromises cardiac function.

Our results implicate the mitochondrial fusion protein Mfn2 as a Parkin receptor on damaged mitochondria, linking mitochondrial regeneration with selective organelle culling. Studies have suggested a role for Mfn2 in mitophagy downstream of Parkin (4, 5), but it also appears to function upstream to help translate the PINK1 signal for

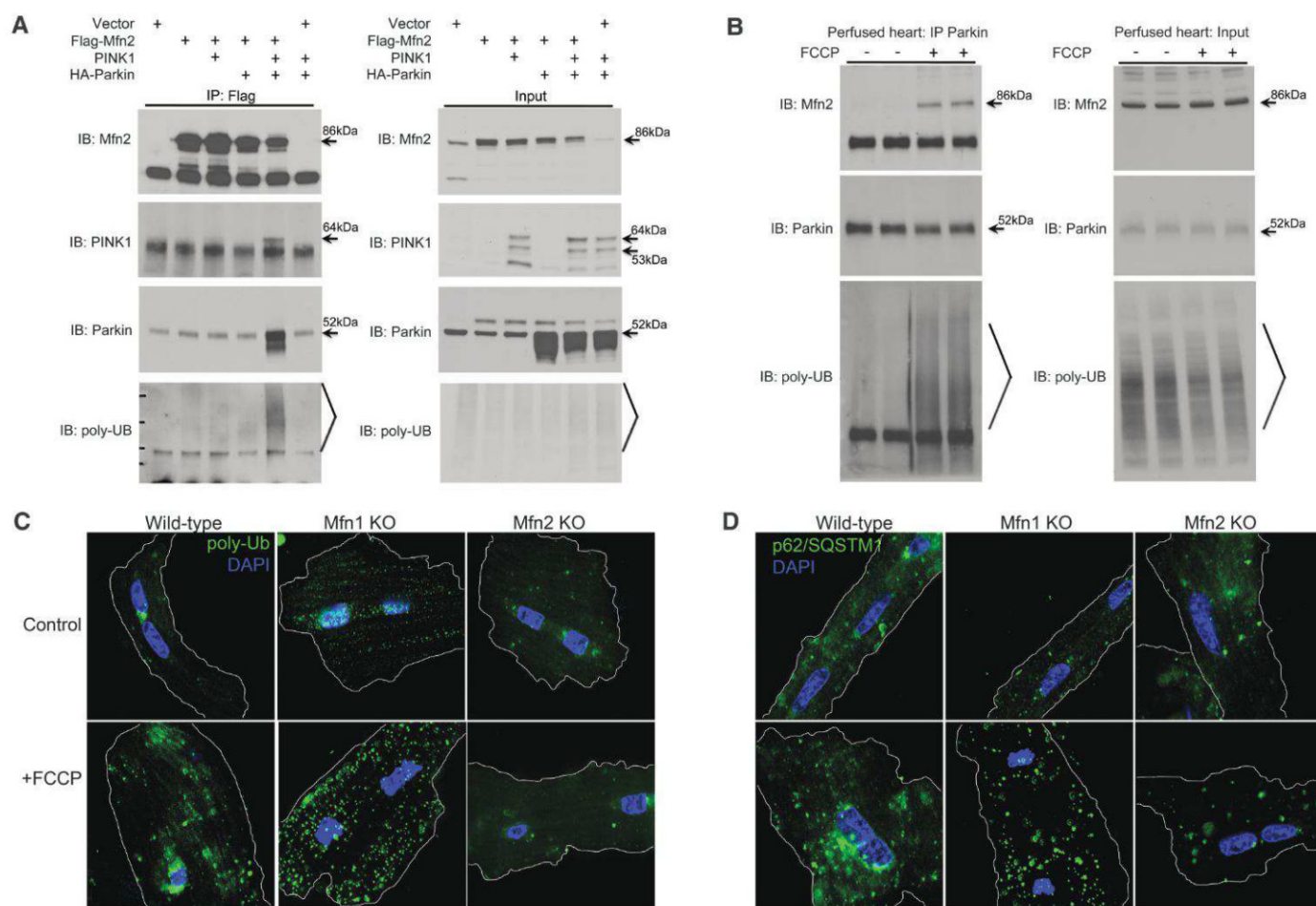
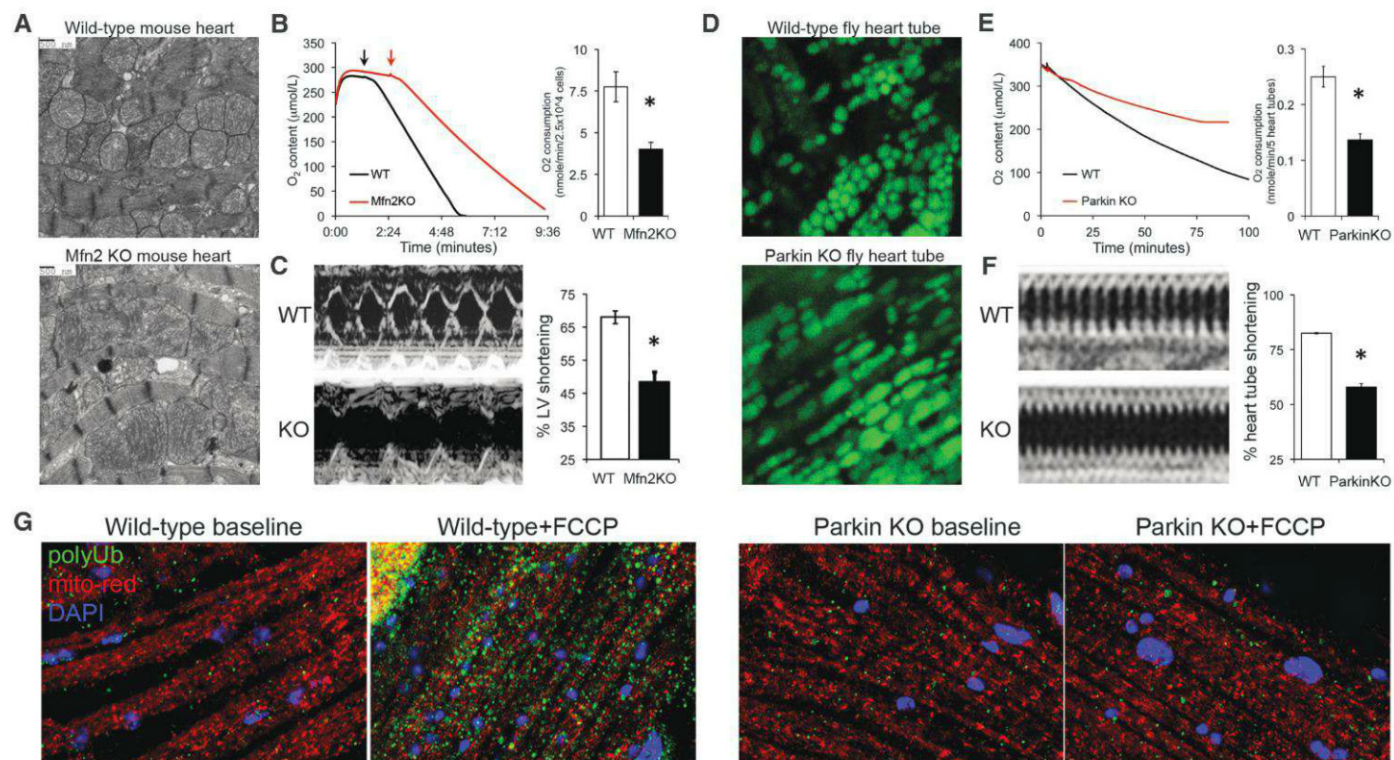
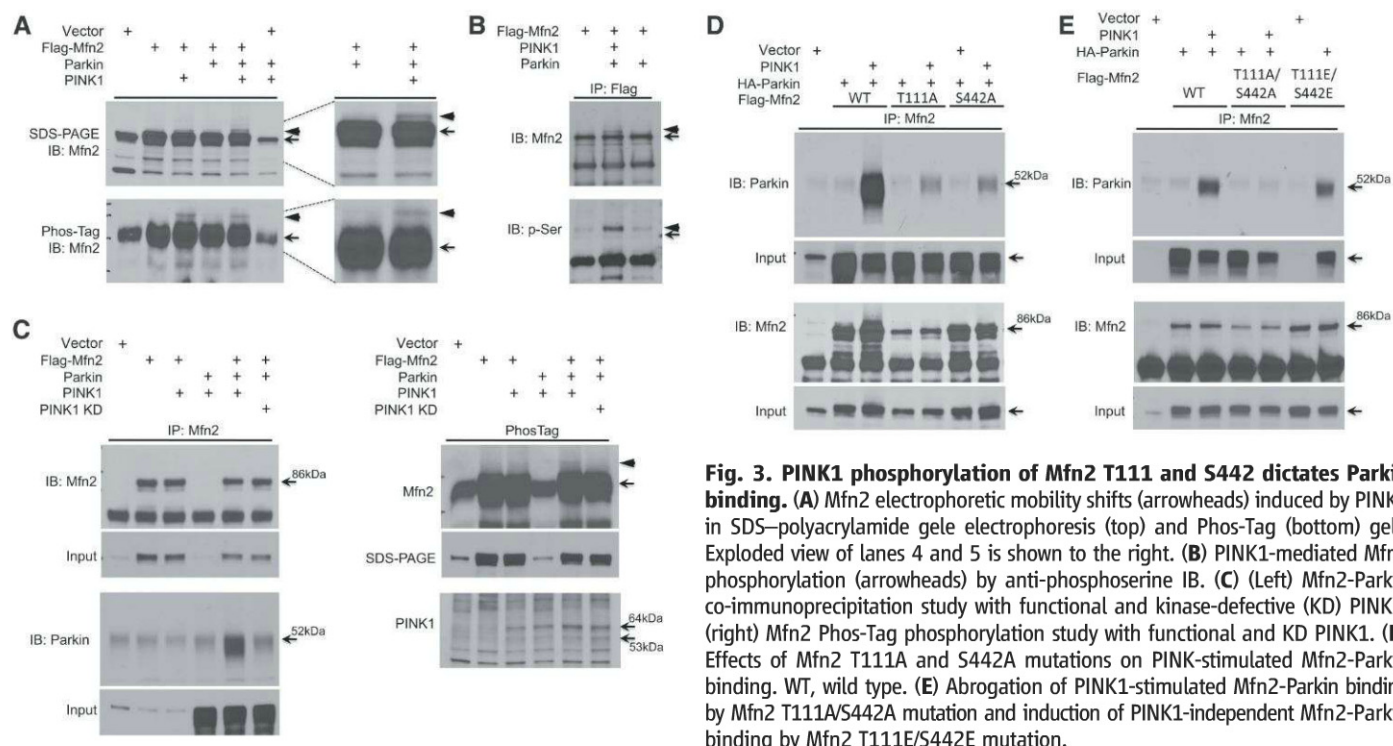


Fig. 2. Defective ubiquitination and mitophagy in Mfn2-deficient mouse hearts. (A) Mfn2 ubiquitination (UB) in PINK1/Parkin-containing HEK cell immune complexes (left). Input proteins are on the right. (B) Protein ubiquitination stimulated by FCCP in Mfn2/Parkin immune complexes from

mouse heart. (C) Mitochondrial ubiquitination (green) induced by FCCP in wild-type and Mfn1- and Mfn2-deficient mouse cardiomyocytes. Cell borders are outlined in white. (D) Parallel studies of mitophagy-adaptor protein p62/SQSTM1 (green).



Parkin translocation. We envision that PINK1 stabilized in depolarized mitochondria phosphorylates Mfn2, which attracts and binds Parkin to promote mitophagy. In the absence of Mfn2, the PINK1-Parkin pathway of mitophagic quality control is interrupted, abnormal mitochondria accumulate, and cardiac toxicity ensues. A broad role for Mfn2 as Parkin receptor is indicated by mitochondrial abnormalities also observed with liver- and neuron-specific Mfn2 ablation (12, 13). Parkin translocation to mitochondria in cultured embryonic fibroblasts lacking Mfn2 (8) indicates activity for one or more compensatory Parkin binding mechanisms, although respiratory compromise suggests that these secondary mechanisms are incompletely effective (fig. S13). Like tethering to endoplasmic reticulum (14), the unique Parkin receptor function of Mfn2 distinguishes it from Mfn1. Defective Parkin binding may have importance in hereditary neuropathies genetically linked to mutations of Mfn2 and characterized by

accretion of abnormal mitochondria (15). The adverse consequences of interrupting Parkin-mediated mitophagy in the heart might also provide insight into the previously unexplained epidemiological link between Parkinson's disease and cardiomyopathy in the elderly (16).

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Supplementary Materials

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Direct Proteomic Quantification of the Secretome of Activated Immune Cells

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Protein secretion allows communication of distant cells in an organism and controls a broad range of physiological functions. We describe a quantitative, high-resolution mass spectrometric workflow to detect and quantify proteins that are released from immune cells upon receptor ligation. We quantified the time-resolved release of 775 proteins, including 52 annotated cytokines from only 150,000 primary Toll-like receptor 4-activated macrophages per condition. Achieving low picogram sensitivity, we detected secreted proteins whose abundance increased by a factor of more than 10,000 upon stimulation. Secretome to transcriptome comparisons revealed the transcriptionally decoupled release of lysosomal proteins. From genetic models, we defined secretory profiles that depended on distinct intracellular signaling adaptors and showed that secretion of many proinflammatory proteins is safeguarded by redundant mechanisms, whereas signaling adaptor synergy promoted the release of anti-inflammatory proteins.

Secreted proteins function as key intercellular messengers in multicellular organisms. Such proteins include cytokines, interleukins, growth factors, hormones, and others that propagate biological information in the body to coordinate homeostasis (1). The investigations of protein secretion have primarily relied on antibody-based methods, whose large-scale use is limited by availability, specificity, and affordability. Strategies for comprehensive, unbiased, and quantitative analysis would therefore be highly desirable, especially for the systems-level characterization of regulated secretory programs (2–5). We therefore developed a sensitive high-resolution mass

spectrometry (MS)-based method to detect proteins in complex secreted protein mixtures with sufficient speed to investigate a large number of conditions. Proteins in supernatants of primary macrophages grown in a 48-well format were digested with trypsin, and the resulting peptide mixtures were directly analyzed in a single-run liquid chromatography mass spectrometry (LC-MS) format (6, 7). We performed LC with 2-hour gradients and analyzed peptides on a benchtop quadrupole-Orbitrap instrument with very high sequencing speed and high mass accuracy in MS and tandem MS (MS/MS) modes (8). Label-free quantification of the MS data and statistical analysis was done in the MaxQuant environment (see the supplementary materials) (9, 10).

Cells of the immune system sense pathogens through specific receptors and release proteins that orchestrate the immune response. The gram-negative bacterial component lipopolysaccharide

(LPS) is a potent activator of protein secretion through the stimulation of Toll-like receptor 4 (TLR4). This membrane receptor signals through the proximal adaptors Toll or interleukin (IL)-1 receptor (TIR) domain-containing proteins MyD88 and TIR domain-containing adaptor-inducing interferon (TRIF) (11, 12). Efficient activation of TLR4 requires the delivery of LPS to the receptor signaling platform by proteins also present in serum (13, 14). Key challenges for MS-based methods in secretome studies are the low abundance of immune-modulatory proteins, such as cytokines, in complex mixtures that typically contain highly abundant components. Therefore, we tested bone-marrow-derived macrophages to functionally induce the secretion of known MyD88- and TRIF-dependent proteins in the presence of sufficiently low, MS-compatible amounts of serum (fig. S1). For cells washed once with serum-free medium, we obtained time-resolved samples of wild-type (WT), MyD88-KO, TRIF-KO, and double-KO (knockout) macrophages treated with or without LPS at five time points in at least triplicates, adding up to more than 120 secretome measurements.

MS analysis (Fig. 1A) confirmed the increased abundance of known cytokines from the cells stimulated with LPS in a time- and genotype-dependent manner, whereas protein groups annotated as cytoplasmic were retained inside the cell under all conditions. Numerous proteins from bovine serum were present in all conditions, indicating that our proteomics workflow remains effective in the presence of low amounts of serum (fig. S2, A and B). We calculated relative-change (fold-change) values for protein abundances in cell supernatants with and without LPS stimulation for each genotype and time point by taking the ratio of the individual protein abundances as described in detail in the supplementary materials. These values correlated well within biological replicates (median $R = 0.84$) and with protein

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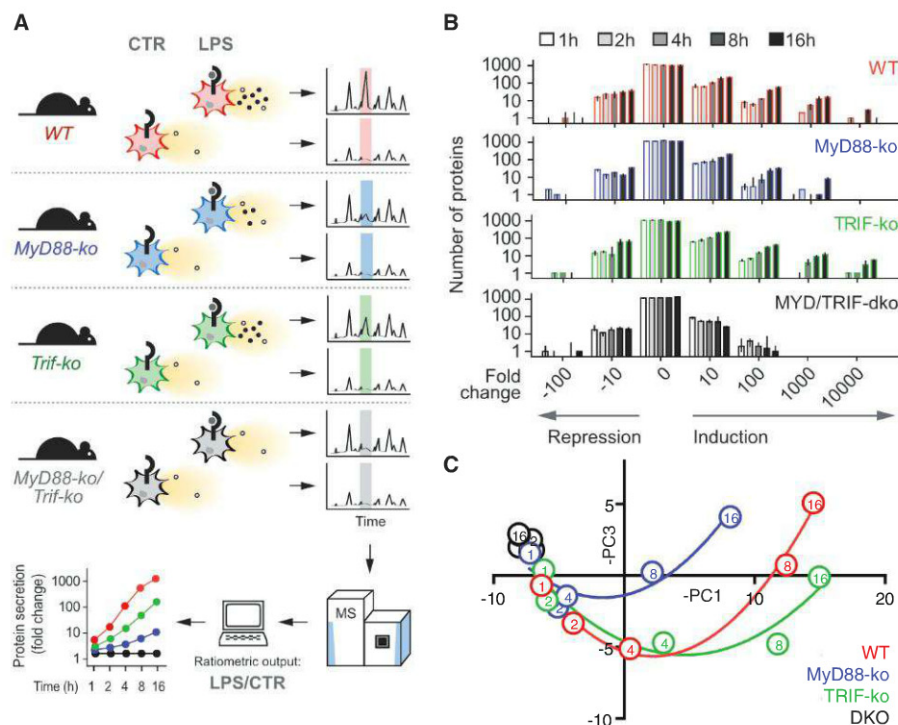


Fig. 1. The TLR4 induced protein secretome. (A) Schematic illustration of the experimental setup, proteomics workflow, and data analysis. (B) Number of proteins with the indicated fold change in release upon LPS treatment. Centers of bins are depicted; bars represent the median of replicates with range. (C) PCA of significantly released proteins plotted as median. Numbers indicate time points after LPS stimulation. R^2 of the second-order polynomial fits of the genotypes over time: WT = 0.99, MYD-ko = 0.83 and, TRIF-ko = 0.79. PCA vectors PC1 and PC3 contributed 41.7% and 5.3%, respectively, to the genotypic variance.

abundance determined by enzyme-linked immunosorbent assay (ELISA) (fig. S2, C to F). MS-based quantitation readily distinguished proteins secreted after receptor ligation from basally secreted or serum-derived ones, which had ratio-metric values close to one. Comparison with ELISA experiments verified that the MS sensitivity was in the picogram range.

Our analysis detected 775 proteins that were reproducibly released by macrophages upon TLR4 induction (Fig. 1B, fig. S2G, and database S1). Among these proteins were 364 proteins with annotated extracellular functions, including 52 known cytokines (table S1 and database S1). A third of the proteins released upon LPS treatment were annotated glycoproteins (fig. S3). For two-thirds of them, there was evidence of signal peptides or membrane localization. This still leaves many proteins that may be released by unconventional secretory mechanisms (15). Our technique may thus be particularly suited for the identification of unexpected extracellular proteins or proteins with unknown routes of cellular exit (16). Many of the released proteins had transmembrane regions and could often be divided into those potentially released by proteolytic cleavage or by shedding of membranes on the basis of the topological localization of the identified peptides in the proteins (fig. S3B). A total of 782 proteins were less abundant after TLR4 activation, but their median change was less than 2-fold and they showed no signaling adaptor-dependent dynamics. Therefore, we conclude that LPS stimulation primarily induces protein secretion (Fig. 1, B and C; figs. S4 and S5; and supplementary text).

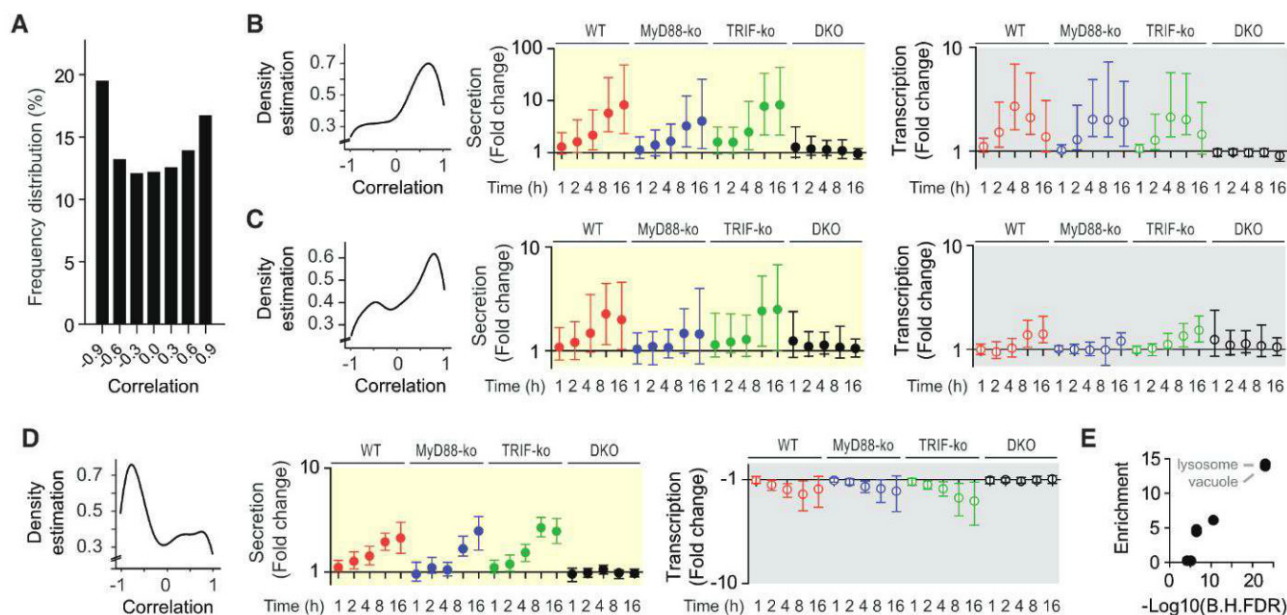


Fig. 2. Relation of transcription to protein secretion. (A) Frequency distribution of correlations for induced protein secretion to transcriptome. Centers of the bins are indicated. (B and C) Proteins with correlated secretory and transcriptional regulations. (B) Secreted proteins accumulate over time; transcripts decline at late time points. (C) Secreted proteins and transcripts accumulate over time. (D) Proteins with anticorrelated secretory and transcrip-

tional regulations. [(B) to (D)] (Left) Density estimation of correlation. (Middle) Median with interquartile range of secreted proteins. (Right) Median with interquartile range of transcriptional regulation. (E) Gene ontology enrichment for cellular component (GOCC slim) of the anticorrelated proteins using the Fisher's exact test on the complete data set, plotted versus Benjamini Hochberg false discovery rate (B.H.FDR) corrected significance.

Our time- and genotype-dependent data showed that the number and individual abundances of secreted proteins increase. It appeared to depend strictly on signal transduction of at least one of the two proximal signaling adaptors, because deficiency in both MyD88 and TRIF (double KO) abolished dynamic LPS-dependent protein secretion (Fig. 1, B and C). Time-resolved principal components analysis (PCA) indicated that the differences in the secretory profiles of the genotypes produced the greatest diversion at 8 hours and 16 hours (Fig. 1C).

Extracellular and secreted proteins undergo a series of posttranscriptional steps to arrive at their site of action, including translation, processing, and transport by secretory machineries. When we measured transcript abundances by microarrays, the measured dynamic range of in-

duction was more than 10 times as high in the MS data. Intriguingly, there were clear differences in the correlation of transcript and secretome changes for different populations of genes (Fig. 2A and fig. S6A). For 290 identified proteins, we observed positive correlations between the regulation of the transcripts and secreted proteins. For some of them, the time-resolved dynamics of the transcripts agreed directly with the secretion, whereas for others, transcript abundance peaked at 4 hours and then decreased while the secreted protein continued to accumulate (Fig. 2, B and C, and fig. S6, B and C). The latter population included many cytokines and secreted proteins, such as interleukin 6 (IL6), C-X-C motif chemokine 2 (Cxc12), plasminogen activator inhibitor 2 (Serpinb2), and serum amyloid A-3 protein (Saa3), but also a number of proteins not

known to be secreted. Unexpectedly, 119 proteins whose secretion was dependent on signal transduction by either MyD88 or TRIF, transcriptome and secretome were negatively correlated (Fig. 2D and fig. S6D). Gene ontology analysis of this class of proteins revealed a strong enrichment for lysosomal content (Fig. 2E) (15, 17). We confirmed the transcriptionally independent release of lysosomal cargo proteins by Western blots and ELISA assays (fig. S7). Based on the observation that proteolytically released (as opposed to membrane-shed) proteins accumulated in the supernatant at later time points of activation, we speculate that proteases, including those of lysosomal origin, may have unanticipated extracellular functions (fig. S3, C and D).

To discover proteins with secretory signatures specific for MyD88 or TRIF, we filtered for proteins that differed significantly in their secreted abundances (fig. S8, A and B) (18). MyD88 accounted for a greater number of more highly secreted proteins than did TRIF, with the highest impact at later time points (Fig. 3, A to D) (19, 20). The proteins whose secretion depended on the adaptors also accounted for much of the secretion in WT cells, with a progressive prevalence of MyD88-mediated signal transduction over time (Fig. 3E and fig. S7, C and E). Differentially secreted proteins included known MyD88-dependent inflammatory cytokines [for instance, growth-regulated alpha protein (Cxc11), tumor necrosis factor (Tnf), C-C motif chemokine 3 (Ccl3)], TRIF-dependent antiviral cytokines [such as interferon beta (Ifnb), C-X-C motif chemokine 10 (Cxc10), C-C motif chemokine 8 (Ccl8)], as well as other cytokines, complement components, protease inhibitors, and acute phase proteins (Fig. 3 C and D) (21). We also quantified proteins as differentially regulated that are not known to be released by macrophages, such as the putative TNF-resistance-related protein U90926, illustrating the potential of our approach to identify proteins with unknown extracellular functions and classify their adaptor dependency.

In the presence of both signaling adaptors, the secretory output is determined by adaptor interplay, which can be synergistic or redundant (22). To quantitatively evaluate regulatory adaptor crosstalk, we filtered for synergistically released proteins by comparing secreted protein abundances in WT cells to the combined abundances of the single-adaptor KO as described in detail in fig. S8 and the supplementary materials. The results provided clear evidence for synergistic and redundant mechanisms of protein secretion, and the effects increased as a function of time of LPS stimulation (Fig. 4, A to C). Redundant mechanisms were the most common mode of regulation; for a multitude of proteins, the increase of secretion in the WT and single-adaptor KO were comparable. This may allow efficient mechanisms to achieve full response regardless of the route that the signal travels. This group included the proinflammatory rather than antiviral proteins (Fig. 4D and fig. S8, D

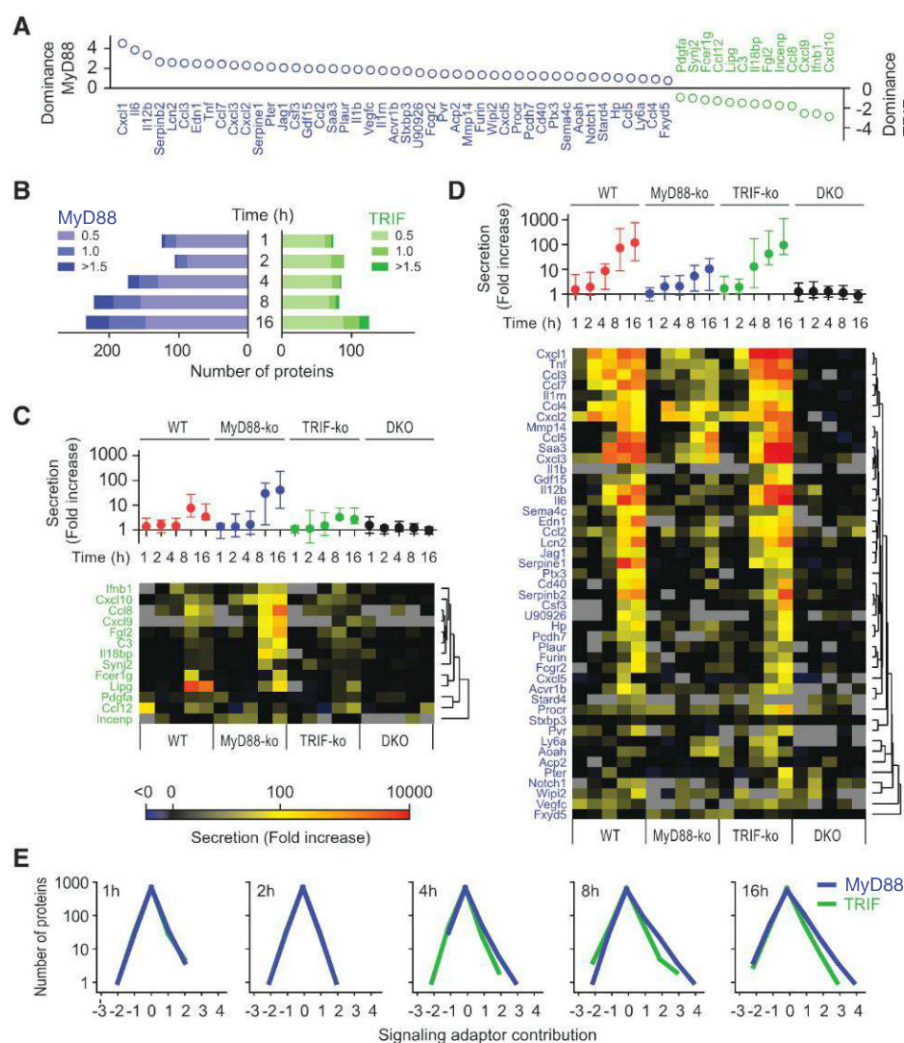


Fig. 3. Effect of signaling adaptors on secretory signatures. (A) Adaptor dominance ranked as maximal difference between MyD88- and TRIF-mediated secretion. (B) Number of proteins predominantly released by signal transduction through MyD88 or TRIF, respectively. Shades indicate the strength of differential regulation in log10. (C) TRIF-dominated and (D) MyD88-dominated protein secretion. (Top) Median with interquartile range for proteins whose secretion depended on the adaptor. (Bottom) Heat map of differentially regulated proteins (gray denotes missing values). (E) Contribution of MyD88 and TRIF to the secretory response induced by both adaptors. Number of proteins plotted versus the strength of the contribution for the indicated genotype.

and G), presumably because robustness of secretion of these proteins is crucial to initiate inflammation.

Proteins that require synergistic action of both adaptors—interleukin-10 (IL10), IL19, metalloproteinase inhibitor 1 (Timp1), endothelial lipase (Lipg), and plasminogen activator inhibitor 1 (Serpin1)—were released only at late time points and were at least 100-fold more strongly secreted if both adaptors were present than if only one

adaptor was present (Fig. 4, D and E, and fig. S8D). IL10 and its family member IL19 have anti-inflammatory properties (23, 24). Because the absence of either of the adaptors prevents their release, increased presence of proinflammatory molecules would be expected at later time points, and this is what our data show (Fig. 4, F and G). Our data suggest that both adaptors are required for establishing regulatory feedback to control inflammation by simultaneously pro-

moting release of anti-inflammatory proteins and preventing excessive release of proinflammatory proteins. We quantified selected proteins and mRNAs with independent techniques and observed excellent agreement (figs. S7 and S9).

In summary, we have described a combination of high-resolution MS, rigorous computational proteomics, and statistical methods to discover significantly secreted proteins in response to receptor ligation that enables a systematic dissection of signaling pathways and the identification of proteins with transcriptionally independent or unexpected extracellular functions.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6131/475/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S9
Table S1
Database S1
References (25–29)

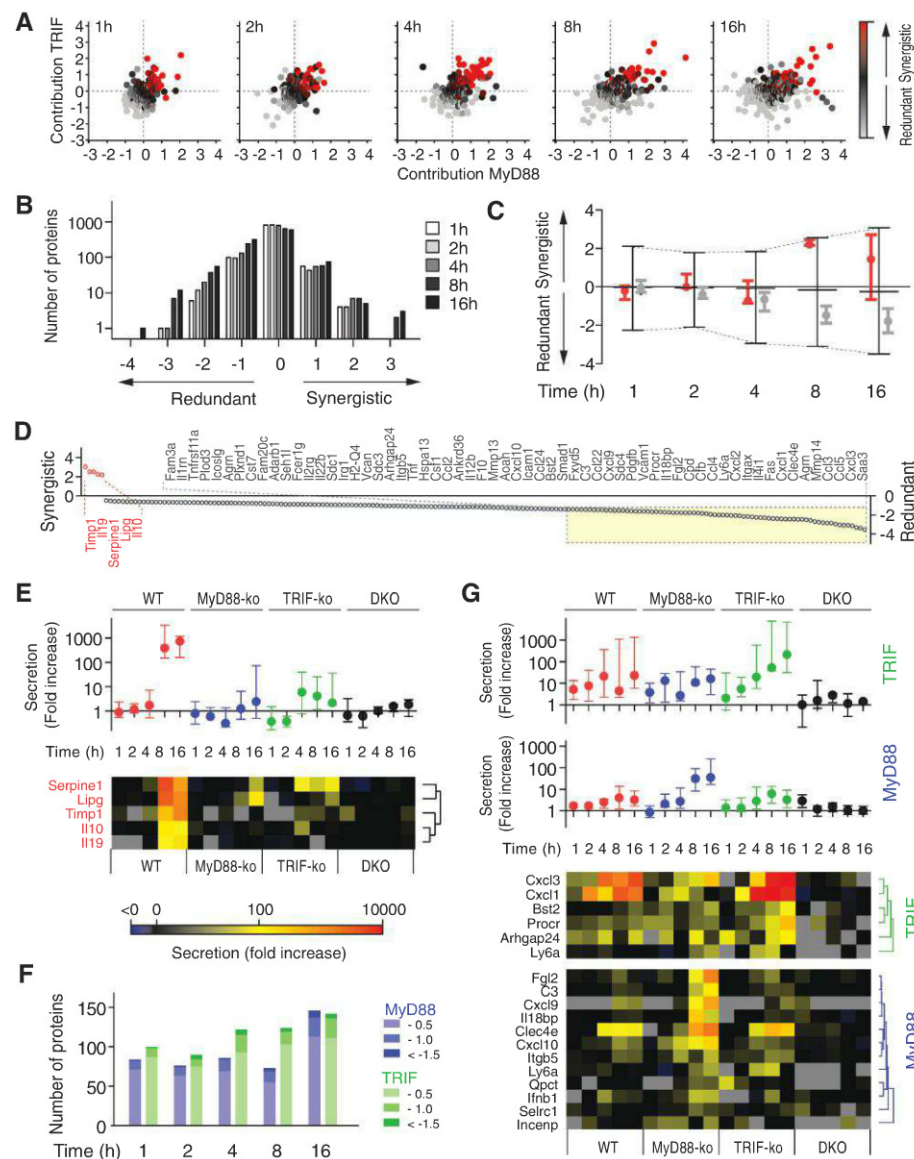


Fig. 4. Signaling adaptor interplay. (A) Contribution of MyD88 and TRIF to the secretory output illustrated for redundant and synergistic adaptor interplay. (B) Number of proteins with redundant and synergistic regulation over time. Centers of the bins are indicated. (C) Progressive adaptor interplay with median and ranges. Trends for synergistic and redundant protein regulations as median with interquartile range are indicated in red and gray, respectively. (D) Adaptor interplay ranked as maximal difference between WT and a combination of MyD88 and TRIF. Proteins with a maximal redundant regulation ≤ -1.5 are shaded. (E) Synergistic protein secretion. (Top) Median with interquartile range of proteins requiring both adaptors for maximal secretion. (Bottom) Heat map of regulated proteins. (F and G) Proteins with increased secretion in the single-adaptor KO compared with WT. (F) Number of proteins with the indicated strength of differential regulation in log10. (G) Protein secretion of MyD88- and TRIF-dependent proteins. (Top) Median with interquartile range. (Bottom) Heat map of regulated proteins.

Deciphering the Glycosylome of Dystroglycanopathies Using Haploid Screens for Lassa Virus Entry

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Glycosylated α -dystroglycan (α -DG) serves as cellular entry receptor for multiple pathogens, and defects in its glycosylation cause hereditary Walker-Warburg syndrome (WWS). At least eight proteins are critical to glycosylate α -DG, but many genes mutated in WWS remain unknown. To identify modifiers of α -DG, we performed a haploid screen for Lassa virus entry, a hemorrhagic fever virus causing thousands of deaths annually that hijacks glycosylated α -DG to enter cells. In complementary screens, we profiled cells for absence of α -DG carbohydrate chains or biochemically related glycans. This revealed virus host factors and a suite of glycosylation units, including all known Walker-Warburg genes and five additional factors critical for the modification of α -DG. Our findings accentuate the complexity of this posttranslational feature and point out genes defective in dystroglycanopathies.

In humans, α -dystroglycan (α -DG) links the extracellular matrix with the cytoskeleton and is extensively modified by sugar chains, including an unusual O-linked glycan (1). Mutations in genes required for α -DG glycosylation lead to congenital disorders, termed dystroglycanopathies. Notable is Walker-Warburg syndrome (WWS) (2), a severe muscular dystrophy with malforma-

tions of the eyes and brain, associated with defective binding of α -DG to its ligands, such as laminin (3). The O-linked carbohydrate unit is also used by pathogens to enter their host, including *Mycobacterium leprae* (leprosy) (4), Lassa virus (LASV), and other Old World arenaviruses (5, 6). At least eight potential glycosyltransferases are required to install the laminin-binding epitope

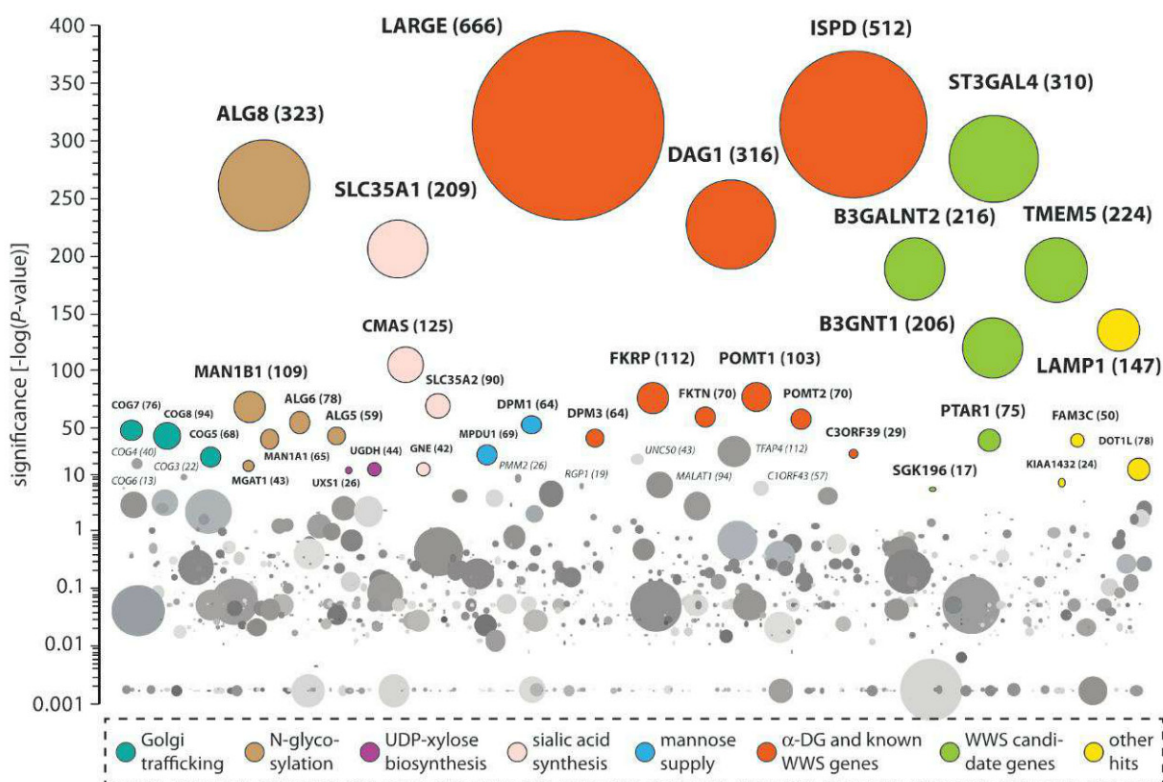
on α -DG (7–9), but only ~50% of the WWS cases are explained by mutations in these genes (8).

We undertook a haploid genetic approach (10) to identify host factors essential for LASV entry. For this purpose, we replaced the glycoprotein of replication-competent vesicular stomatitis virus (VSV) with the Lassa virus glycoprotein (rVSV-GP-LASV) (fig. S1A). This virus infects normal human fibroblasts, whereas patient fibroblasts carrying mutations in the WWS gene *ISPD* (isoprenoid synthase domain containing) (8, 9) resist infection (fig. S1B). Likewise, haploid

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Fig. 1. Haploid genetic screen for cellular host factors required for rVSV-GP-LASV infection. Significance of enrichment of gene-trap insertions in the virus-selected population compared with nonselected control cells is indicated on the y axis. Bubbles represent genes, and bubble size corresponds to the number of independent gene-trap events observed in the virus-selected population. Significant hits are grouped by function horizontally (other genes in random order). Genes carrying the majority of gene-trap insertions in introns were colored if they passed two statistical tests: enrichment of disruptive mutations compared with control cells (one-sided Fisher's exact test, $P \leq 10^{-5}$) and bias for gene trap–insertion events in the transcriptional orientation of the affected gene (binomial test, $P \leq 0.05$). Intron-poor genes were colored if they passed the former criterion using a stricter cut-off (one-sided Fisher's exact test $P \leq 10^{-30}$) (13). Data are displayed until $-\log(P \text{ value}) = 0.001$.



human HAP1 cells (11) are also infected and killed in an α -DG-dependent manner (fig. S2, A and B).

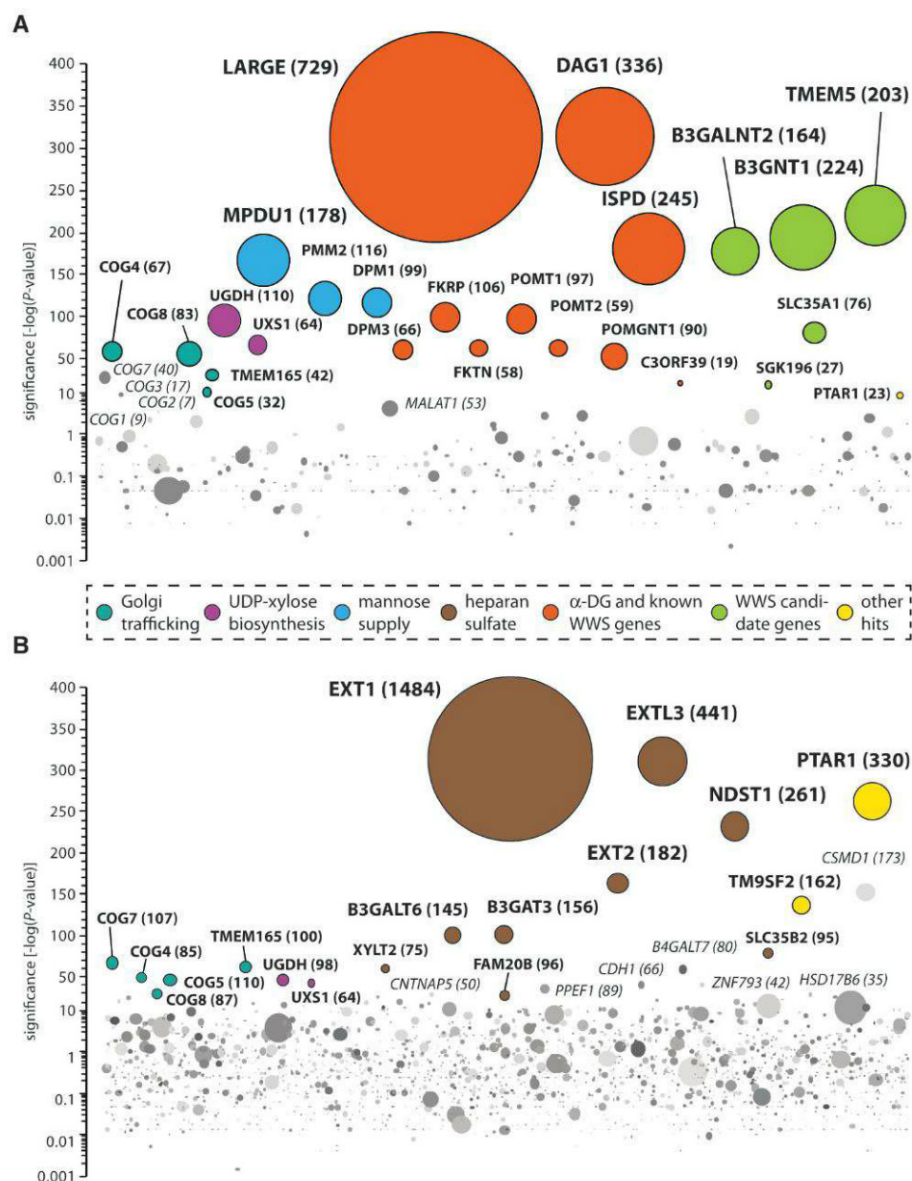
Mutagenized HAP1 cells were exposed to rVSV-GP-LASV, and gene trap–insertion sites were analyzed in virus-resistant cells (12). Genes significantly enriched for mutagenic gene trap–insertion events include *DAG1*, encoding α -DG, with 316 independent disruptive gene-trap insertions and 25 other genes that are predicted or known to be involved in glycosylation (Fig. 1 and figs. S3 and S4) (13). Among these are *LARGE*, *ISPD*, *FKTN*, *FKRP*, *POMT1*, *POMT2*, *DPM3*, and *C3orf39*, all of which cause dystroglycanopathies (2, 7, 14), and *B3GNT1*, which was uncovered as a new WWS gene during preparation of this manuscript (15). Other hits include genes involved in sialic acid biosynthesis, the generation of uridine diphosphate (UDP)–glucuronic acid and UDP-xylose, N-glycosylation, mannose supply, and localization of glycosylating enzymes in

the Golgi apparatus. Last, we found a number of potential enzymes that have not been linked to α -DG modification before (*SGK196*, *TMEM5*, *PTAR1*, *ST3GAL4*, and *B3GALNT2*) and hits that did not readily connect to glycosylation. None of the enriched genes were identified when mutagenized HAP1 cells were selected with a recombinant VSV carrying the Ebola virus glycoprotein (11), which suggested that they are not required for biology related to the VSV vector. Thus, the haploid screen identifies host factors required for virus entry mediated by the Lassa glycoprotein, including the known entry receptor, known receptor modifiers, and a substantial number of additional genes.

As virus entry is a complex succession of events, we teased apart the roles of the identified genes through a series of comparative genetic screens. Principally, hits could have a specific role in α -DG glycosylation, they could affect glycosylation in general, or they could act in virus

entry steps unrelated to receptor binding. We enriched mutagenized HAP1 cells for defective presentation of glycosylated α -DG at the cell surface (fig. S5, A to C). This population showed a significant increase for haploid cells carrying gene-trap insertions in all known WWS genes, indicating that this mutagenesis screen was carried out at high coverage (Fig. 2A). Genes required for N-glycosylation and sialic acid biosynthesis were not enriched, in line with the notion that the laminin-binding epitope on α -DG is created through O- rather than N-glycosylation (1) and does not require the presence of sialic acid (16). An unexpected exception to this is *SLC35A1*, which encodes a transporter for cytidine monophosphate (CMP)–sialic acid (17, 18). This may indicate that this gene is involved in the transport of other sugars needed for α -DG O-glycosylation or that it indirectly affects generation of the laminin-binding epitope. Together, this screen identifies genes required for α -DG modification and dis-

Fig. 2. Cell surface profiling of mutagenized haploid HAP1 cells. (A) Genes enriched for mutations in a cell population depleted for glycosylated α -DG at the cell surface. The cell population enriched for mutants lacking glycosylated α -DG at the cell surface was analyzed and depicted as described in Fig. 1. **(B)** A mutant cell population selected for diminished cell surface heparan sulfate was obtained as described above. Data were analyzed as previously, except that, for intron-rich genes, the cut-off for disruptive mutations compared with control cells was adjusted (one-sided Fisher's exact test, $P \leq 10^{-21}$) (13).



tinguishes them from host factors mediating LASV entry unrelated to α -DG binding (e.g., *LAMP1* and genes required for sialic acid biosynthesis).

To distinguish general glycosylation genes from those required specifically for the generation of the laminin-binding epitope on α -DG, we probed mutagenized HAP1 cells for defects in the generation of heparan sulfate in a separate genetic screen (Fig. 2B and fig. S6). The carbohydrate chains present on α -DG or in heparan sulfate are both thought to contain xylose and glucuronic acid moieties, and indeed, genes required for their biogenesis (*UGDH* and *UXS1*) also stood out in this screen (19, 20). Other overlapping hits affect glycosylation globally, such as the COG complex and *TMEM165* (21). PTAR1 constitutes a potential prenyltransferase that has not been implicated in glycosylation before but also appears to affect glycosylation globally (fig. S4). Finally, cells depleted for heparan sulfate on their surface were enriched for mutations in heparan sulfate biosynthesis genes (Fig. 2B and fig. S4) (19). This finding suggests that although there are biochemical similarities between heparan sulfate and the O-carbohydrate chains on α -DG, these are, by and large, installed by separate enzymes.

Using transcription activator–like effector nucleases (TALENs), we generated null alleles for a panel of selected genes in HAP1 cells (fig. S7) (22), and independent clones were isolated carrying frameshift mutations and/or premature stop codons (Fig. 3A and fig. S8). TALEN-induced mutations in all genes except for *ST3GAL4* and *LAMP1* affected α -DG glycosylation or its ability to interact with laminin (fig. S9, A to C, and fig. S10, A and B). This is in agreement with the absence of *ST3GAL4* and *LAMP1* as hits in the α -DG antibody screen (see Fig. 2A and fig.

S4). Mutant cell lines also showed increased resistance to viral infection, although this phenotype was less pronounced in the *SGK196* mutants (Fig. 3B and fig. S10C). TALEN-induced phenotypes were reverted by complementation with the respective cDNAs (fig. S11, A and B). In summary, we conclude that *TMEM5*, *B3GALNT2*, *B3GNT1*, *SLC35A1*, and *SGK196* constitute genes required for the presentation of the laminin-binding carbohydrate feature present on α -DG, whereas *ST3GAL4* and *LAMP1* are likely involved in virus infection by means other than modification of α -DG.

TMEM5 encodes a transmembrane protein that has not been assigned any function but that contains an exostosin family domain (*E* value 0.0002) (fig. S12) that is also present in the heparan sulfate biosynthesis enzymes EXT1, EXT2, and EXTL3. *SGK196* contains a kinase-like domain, and knockout mice develop hydrocephalus (23), reminiscent of the brain abnormalities observed in WWS patients. We sequenced the coding exons of *TMEM5* and *SGK196* in a panel of 28 patients with severe dystroglycanopathy, diagnosed with WWS or muscle-eye-brain disease (MEB), not carrying mutations in any known Walker-Warburg gene. Two families with patients that carried homozygous mutations in *TMEM5* were identified. One mutant allele results in a stop codon at position Arg³⁴⁰ [1018(C→T)]; the other family transmits an early frameshift mutation A47Rfs*42 [139(delG)] (Fig. 4A). The male patient with the Arg³⁴⁰* mutation died at the age of 22 months and had clinical manifestations suggestive of WWS (13). The female siblings carrying the frameshift mutation had a slightly milder phenotype suggestive of MEB. A cranial magnetic resonance image (MRI) of one of the af-

fected girls recorded at the age of 1 year showed brainstem atrophy, dilated ventricles, widespread pachygyria, and substantial white matter involvement (Fig. 4B). During revision of this manuscript, mutations in *TMEM5* have also been found in fetuses displaying cobblestone lissencephaly (24). A patient with compound heterozygous mutations L137R and Q258R in *SGK196* and typical WWS phenotype was identified in another family (Fig. 4C). To test causality of the identified mutations for the disease, we supplied HAP1 cells deficient for either *SGK196* or *TMEM5* with cDNAs encoding the patient-derived variants. Unlike their wild-type counterparts, these neither restored α -DG glycosylation (Fig. 4D) nor enhanced susceptibility to infection with rVSV-GP-LASV (fig. S13). Together, the detection and functional validation of *TMEM5* and *SGK196* loss-of-function mutations in families with WWS-MEB-type dystroglycanopathy underlines the relevance of the identified α -DG modifiers for human disease.

For decades, genes associated with Mendelian disorders have been discovered by studying pedigrees of affected individuals. Although expedited by robust sequencing strategies, the identification of causative mutations in genetically heterogeneous conditions remains problematic. Here, we apply a haploid genetic approach to capture the complexity of a severe hereditary disease in vitro. The resulting “glycosylome” of α -DG highlights the intricate nature of this post-translational modification and identifies additional genes mutated in Walker-Warburg syndrome. Because polymorphisms associated with the human *LARGE* gene are under selective pressure in areas where LASV is endemic (25), it becomes of interest to examine the glycosylome genes in virus-exposed populations.

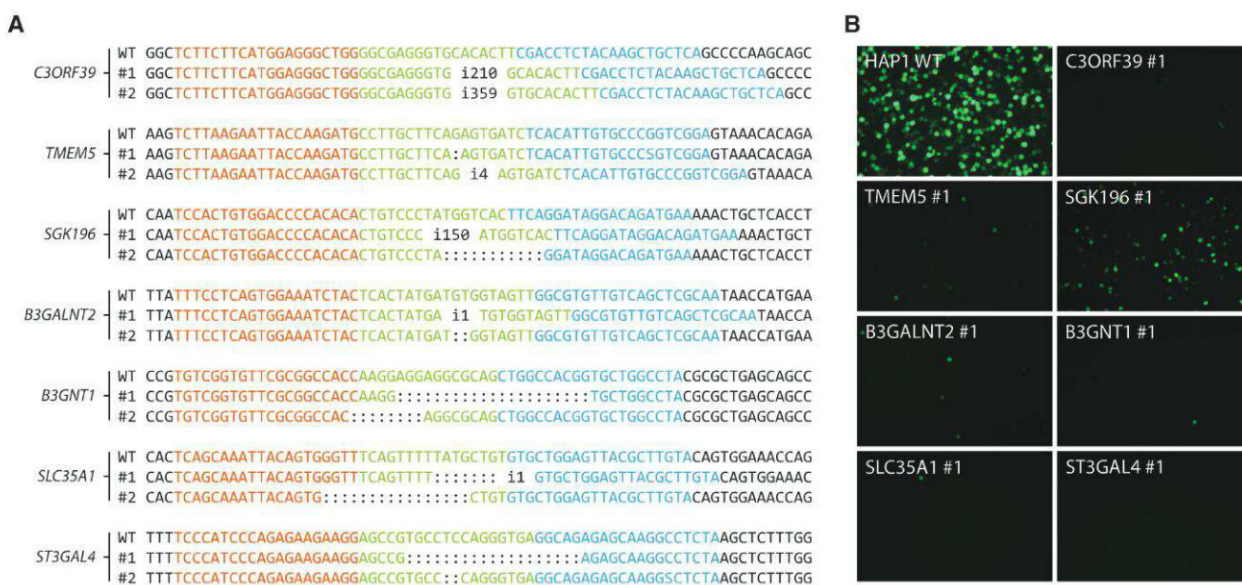


Fig. 3. TALEN-induced mutations in identified genes affect susceptibility to rVSV-GP-LASV. (A) HAP1 cells transfected with TALENs display frameshift mutations and/or introduce premature stop codons in targeted genes. Sequences recognized by the TALENs are displayed in red

and blue. **(B)** The HAP1 cell lines with TALEN-induced mutations in the corresponding genes and wild-type control cells were infected with rVSV-GP-LASV [infected cells express enhanced green fluorescent protein (eGFP)].

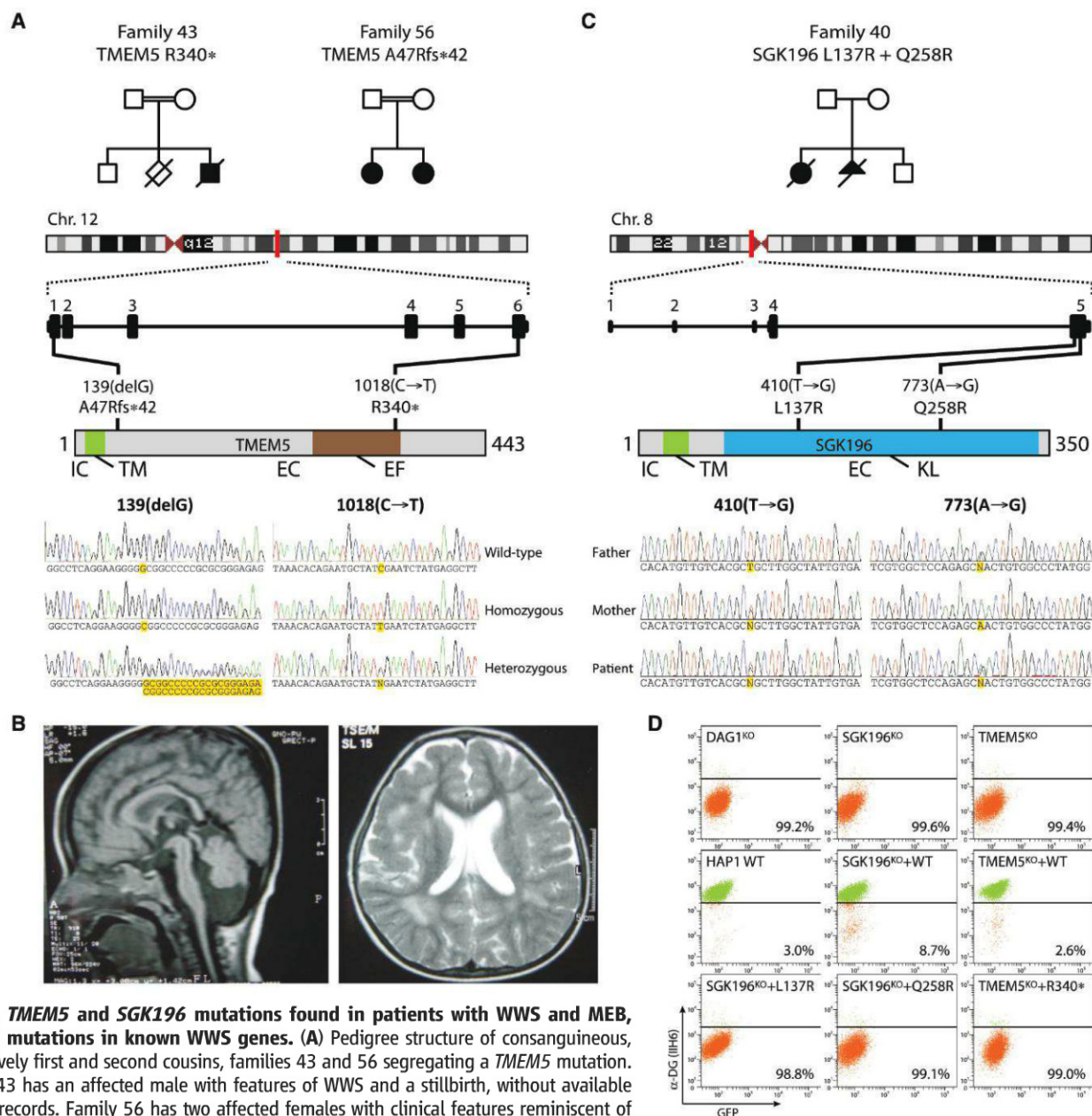


Fig. 4. *TMEM5* and *SGK196* mutations found in patients with WWS and MEB, lacking mutations in known WWS genes. (A) Pedigree structure of consanguineous, respectively first and second cousins, families 43 and 56 segregating a *TMEM5* mutation. Family 43 has an affected male with features of WWS and a stillbirth, without available clinical records. Family 56 has two affected females with clinical features reminiscent of MEB (13). A nonsense mutation in exon 6 was identified in family 43. Family 56 harbors a frameshift mutation in exon 1. Both mutations were homozygously present in the patient(s) and heterozygously in the parents. The unaffected boy in family 43 is heterozygous for the mutation. IC, intracellular domain; TM, transmembrane domain; EC, extracellular domain; EF, exostosin family domain. (B) Cranial MRI of the oldest affected female of family 56 at the age of 1 year; sagittal cut (T1-weighted image): atrophy of pons and cerebellum; axial cut (flair image): fronto-parietal pachygyria, enlarged ventricles, and abnormal white matter. (C) Compound heterozygosity of mutant *SGK196* in an affected patient. Both non-consanguineous parents are heterozygous carriers of either mutation. KL, kinaselike domain. (D) HAP1 cells with TALEN-induced disruption of endogenous *TMEM5* or *SGK196* were complemented with cDNAs encoding the mutant variants observed in patients and analyzed for presence of the α -DG laminin-binding epitope using flow cytometry.

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reverse-genetics system for VSV (International Patent no: 5,789,229), J.E.C. and T.R.B. are inventors on a patent on mutagenesis in haploid or near-haploid cells (U.S. Patent Application no: 2012/0190,011), and materials will be made available to the academic community under a Materials Transfer Agreement. The study was approved by the ethical board of the Radboud University Nijmegen Medical Centre, Commissie Mensgebonden Onderzoek Regio Arnhem-Nijmegen Approval 2011/155 (9612-1812). Deep sequencing data have been deposited in the NCBI Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) under accession number SRP018361.

Supplementary Materials

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Potent Social Learning and Conformity Shape a Wild Primate's Foraging Decisions

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Conformity to local behavioral norms reflects the pervading role of culture in human life. Laboratory experiments have begun to suggest a role for conformity in animal social learning, but evidence from the wild remains circumstantial. Here, we show experimentally that wild vervet monkeys will abandon personal foraging preferences in favor of group norms new to them. Groups first learned to avoid the bitter-tasting alternative of two foods. Presentations of these options untreated months later revealed that all new infants naïve to the foods adopted maternal preferences. Males who migrated between groups where the alternative food was eaten switched to the new local norm. Such powerful effects of social learning represent a more potent force than hitherto recognized in shaping group differences among wild animals.

Ever since pioneering studies on the diffusion of a new sweet-potato washing habit in Japanese macaques (1) and milk-bottle opening in great tits (2), accumulating field studies have suggested that the cultural transmission of behavior through social learning provides many animals with a “second inheritance system” (3). This system complements genetic inheritance and individual learning in shaping behavioral repertoires (4, 5). The scope and impact of this second system are important to delineate because exploiting the existing knowledge of others can potentially support efficient adaptation to local circumstances (6). It can also generate locally differentiated behavioral traditions, and indeed, much of the evidence for a role for animal culture in the wild derives from identifying local variations consistent with the existence of such traditions (7–9). However, owing to their observational nature, these studies lack the experimental rigor to confirm whether putative cultural variations are socially learned. Experiments with captive populations, by contrast, have seeded different groups with models trained to act in different ways, such as opening an “artificial fruit” using either of two alternative techniques, then documenting the dif-

ferential diffusion of these variants across groups (10) and even between them (11).

These paradigms have now produced a substantial corpus of laboratory studies documenting cultural transmission in taxa as diverse as insects (12), fish (13), and apes (11, 14). Such experiments in the wild remain scarce (10, 15–19), however, because in natural populations, it is typically impractical to isolate individuals for differential training as models. The few field studies that have attempted to approximate this approach have generally produced evidence for weaker transmission of the seeded alternatives (15–18) than counterparts in captive populations (10, 11, 20, 21).

Here we introduce a different methodological approach, which has demonstrated two potent effects of social learning in the wild. Instead of seeding behavioral variants in single models, we seeded variants in four whole groups of wild vervet monkeys, *Chlorocebus aethiops*, totaling 109 individuals (22) (table S1 and fig. S1). We then investigated how two classes of individuals naïve to the local group norm—infants and immigrant males—responded to the particular local preferences they were exposed to. To create initial preferences, we provisioned groups with two adjacent trays of maize corn, one with corn dyed blue, the other pink (Fig. 1). One of these (pink in two groups, blue in two others) was made highly distasteful so that after three monthly training sessions, the distasteful alternative was rarely eaten or even tried (table S2 and figs. S3 and S4). After a period of more than 4 months in which a new cohort of identifiable infants matured sufficiently to take solid foods, we again offered the two colored foods with no distasteful treatments and tested (i) whether the naïve infants would copy their mother's preference for the locally favored color over the now equally palatable alternative, and (ii) whether males migrating from a group trained to prefer one color to a second group where the alternative color was preferred would be influenced by the latter.

When the corn provisions were offered again after 4 to 6 months, a preference for the earlier palatable alternative was maintained across five test trials spanning 2 months, despite both colors now being palatable. Some of the previously distasteful food was tried and consumed (Fig. 2 and



Fig. 1. Experimental setup illustrating preferential foraging. Maize corn dyed either pink or blue was provided intermittently in two adjacent containers. Photograph shows infant sitting on the color earlier made distasteful to its mother, as it eats the color currently preferred by its mother and the rest of the group.

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table S3B), but typically only by lower-ranking individuals when they were unable to access the preferred color (table S4), with the result that at least 73% of the corn eaten in any trial was of the trained color (Fig. 2). This persistence of the induced preference over our five trials is important because new subjects joined the experiments up to trial 5.

Of 27 infants naïve to the corn provisions, 26 first ate the locally preferred color eaten by their mother, ignoring the directly adjacent alternative color (some even sat on the nonpreferred color corn, to eat the favored one: Fig. 1). Just one infant's first choice was of the alternative-colored food. Its mother was of low rank and took a small amount of the nonpreferred food while higher-ranked individuals were monopolizing the preferred food. Accordingly, 27 of 27 infants first took the food they witnessed their mothers eat (for 26 of them, the one they would also see most eaten in their group). This represents a highly significant bias that unambiguously demonstrates copying of the local preference (binomial test, $P < 0.0001$), despite the immediate availability of the alternative option. All 23 of such infants who were later recorded eating when their mothers were not at the box continued to match her preference, confirming that their preferred food choice was a result of social learning.

Fifteen males immigrated into the study groups during the experiment (Fig. 3 and fig. S2). Ten were known to us and fortuitously moved to a group that preferred the alternative colored food to that eaten in their original group (Fig. 3 and fig. S2). The first choice of seven of these males, on approaching the food provisions after observing others feeding there, was for the locally preferred option new to them, contrasting markedly with the 11 resident males, none of whom chose their formerly distasteful option (choices 7 of 10 versus 0 of 11: Fisher's exact test, $P = 0.001$, Fig. 3). When each of the 10 migrants first fed with no monkey higher ranking than themselves present, their preference for the locally consumed color was even more pro-

nounced (choices 9 of 10 versus 0 of 11: Fisher's exact test, $P < 0.0001$). The single male (Lekker) who continued to eat the same color as in his original group was unique in immediately taking the top rank in his new group, a factor that may have influenced his behavior. Five additional males migrated into our groups from unknown origins and so were naïve to both colors of corn. The first choice of all of these when no higher-ranking monkey was present was for the local norm. Conservatively testing the preference of all 15 migrant males for the local preference against a random choice confirms a significant positive bias (i.e., 14 of 15: binomial test, $P = 0.001$). Additional tests extending to later feeding on the different options are in the supplementary text and converge with the results above in demonstrating conformity in the migrants' foraging decisions.

Our demonstration of such strong conformity in wild primates has important implications for our conceptions of what is unique in human cultural transmission (23–27) as well as in evolutionary biology more broadly, concerning the roles of cultural versus genetic inheritance and individual learning in shaping adaptations. First, our results reveal a potent role for social learning in both infants and migrating adult primates, which for the latter group extends to social conformity, defined in human studies by the flexible subjugation of prior personal knowledge to the observed habits of a majority of the relevant new community (28) (see supplementary text for more on definitions of conformity) (Fig. 3). Laboratory studies have begun to suggest a role for such conformity in taxa ranging from *Drosophila* to chimpanzees (12, 14, 29–31), but in the wild such evidence has remained minimal and circumstantial (32–34).

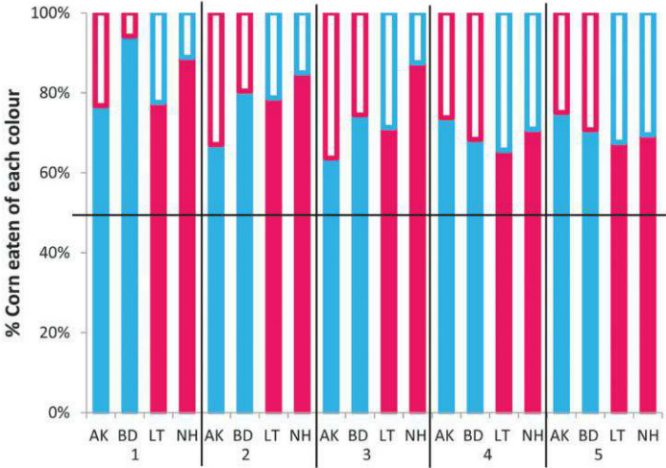
Theoretical work has suggested that reliance on such social information at the expense of personal information can be adaptive because it exploits the fruits of other individuals' prior experience (6, 23, 27). The actions of both the naïve infants and migrating males whom we studied is consistent with such a “copy when un-

certain” strategy. The fitness of foraging decisions made by wild primates like those we studied will be governed by a host of complex factors that are inherently unknown to foragers, ranging from dietary constituents to plant toxins and competing needs such as predator vigilance: Exploiting the prior discoveries of local experts may be an optimal strategy, overriding opposing knowledge gained in a different habitat such as one's original group.

A second implication of our results is methodological. We have simulated the effect on social learning of already well-established traditions by initially creating habits in whole groups, then recording the responses of infants and migrants naïve to these habits. This has revealed very strong effects, contrasting with the relatively weak or nonexistent effects of seeding alternative behavior patterns in single individuals, found in other recent field experiments (15–18). Our new approach thus could be applied more widely to other taxa in the wild, as well as in captive studies.

The final implication arises from the combination of the two already outlined. If copying the majority is indeed a more widespread strategy

Fig. 2. Local food preference norms of four groups. Percentage of each color of food eaten in each group across the five test trials separated, respectively, by intervals of 1, 1, 2, and 4 weeks. Pink, pink dye; blue, blue dye. Full color bars, food color always palatable; white color bars, food color made unpalatable 4 to 6 months earlier. Letter codes represent the four groups (AK, Ankchase; BD, Baie Dankie; LT, Lemon Tree; NH, Noha).



ID	origin	new group	
EI	LT	AK	
Th	LT	AK	
Ar	LT	AK	*
Qu	NH	AK	
Bo	?	AK	

Er	BD	NH	
Le	BD	NH	
Gr	BD	NH	
Mf	AK	LT	
Ge	AK	LT	*
Iz	AK	LT	
Au	?	NH	*
Ch	?	NH	
Sh	?	LT	*
Am	?	LT	

Fig. 3. Males abandon food preference learned in home group in favor of local preference in new, adopted group. Columns show food color preference of each male in original group (origin) and contrasting first choice of food color in adopted new group (framing lines represent preference in adopted group). Asterisk (*) denotes choice of color eaten first once not outranked by residents of adopted group. ID, male identity code.

among wild, nonhuman animals than previously appreciated, the initial establishment of behavioral innovations may be a fragile process, in comparison to the transmission of already-common behaviors. If so, this argues not only for the extension of our group-training approach, but also for systematic comparisons between such experiments and those that instead seed new behaviors in individuals, in order to understand why some innovations are short-lived whereas others spread to become new traditions.

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Supplementary Materials

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Network-Based Diffusion Analysis Reveals Cultural Transmission of Lobtail Feeding in Humpback Whales

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We used network-based diffusion analysis to reveal the cultural spread of a naturally occurring foraging innovation, lobtail feeding, through a population of humpback whales (*Megaptera novaeangliae*) over a period of 27 years. Support for models with a social transmission component was 6 to 23 orders of magnitude greater than for models without. The spatial and temporal distribution of sand lance, a prey species, was also important in predicting the rate of acquisition. Our results, coupled with existing knowledge about song traditions, show that this species can maintain multiple independently evolving traditions in its populations. These insights strengthen the case that cetaceans represent a peak in the evolution of nonhuman culture, independent of the primate lineage.

Debate about traditions and culture in nonhumans has been fueled by claims of evidence for culture, broadly defined as shared behavior propagated by social learning (1), in a variety of species (2–5), including cetaceans (6). Quantifying cultural transmission in any wild population is difficult, however, because field studies are rarely sufficient to allow for the complete elimination of alternative ge-

netic or ecological explanations (1, 7–9). These problems are exacerbated by the limitations on visibility and accessibility inherent in studying marine mammals, but the group continues to attract interest due, for example, to the strong evidence for cultural transmission of vocal patterns (10, 11). Only a handful of cetacean species have lent themselves to the types of data collection necessary to address questions of cultural transmission (6), and the evidence remains, for the most part, controversial (1).

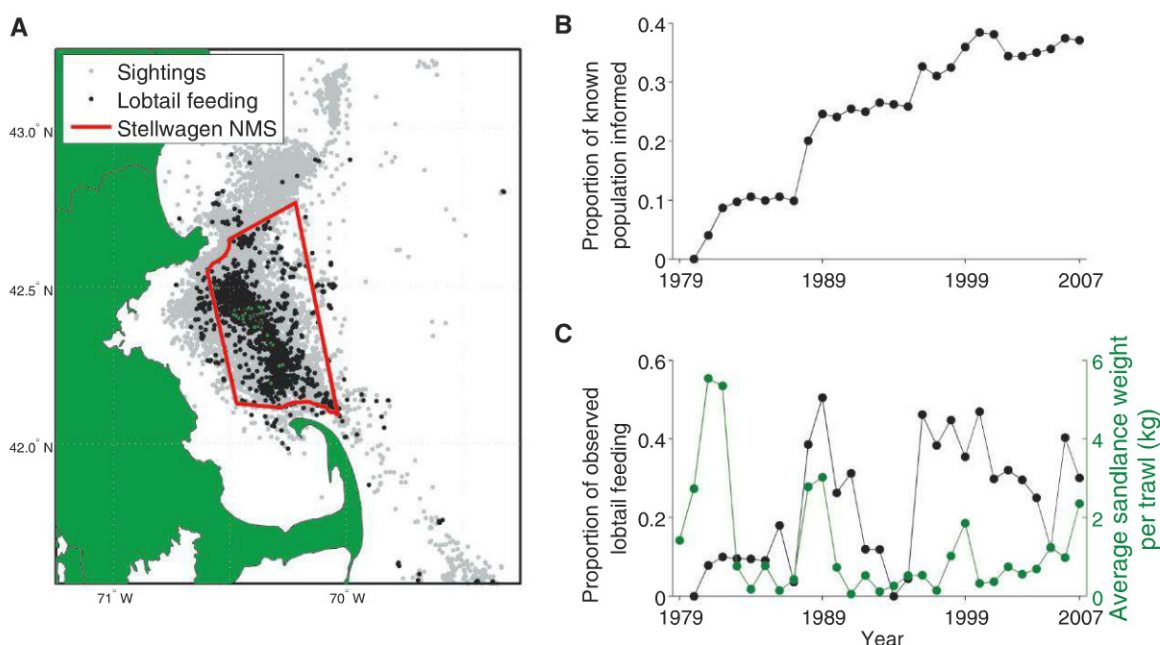
In the Gulf of Maine, bubble-feeding is a common foraging technique used by humpback whales (*Megaptera novaeangliae*), which is characterized by bubble production 20 to 25 m below the surface, underneath and around a prey

school, followed by a lunge through those bubbles (12, 13). Although this general technique has been documented in other humpback populations (14), in 1980 one whale in the Gulf of Maine was observed performing an innovative modification to this feeding technique that is now known as lobtail feeding (13, 15). Lobtail feeding consists of striking the water's surface one to four times with the ventral side of the fluke, followed by a bubble-feeding sequence. An accelerating rate of diffusion of this behavior, indicated by a sharp increase in the proportional use of lobtail feeding in the years 1981–1989 (Fig. 1), led to the suggestion that social transmission was responsible for its spread (15). Diffusion rate, however, is not a reliable indicator of social transmission (16). Furthermore, it has been suggested that lobtail feeding is a specialization related to foraging on sand lance (15), because it is spatially concentrated on Stellwagen Bank (Fig. 1A), where sand lance gather for spawning (17). In the years immediately preceding the behavior's emergence, the stock of herring, another important prey species, crashed (18), suggesting a role for ecological factors. Because such factors are a common influence on innovation and social learning, these hypotheses are not necessarily mutually exclusive, but it is difficult to measure the relative influence of social and ecological factors on the spread of behavior (1, 9). Network-based diffusion analysis (NBDA) (9, 19, 20), a new method related to network influence models in the social sciences, offers one way forward [see also (5, 21)]. We used NBDA to analyze the spread of the lobtail feeding innovation among humpback whales summering in the Gulf of Maine.

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Fig. 1. Spatial (A) and temporal (B and C) distribution of lobtail feeding. (B) shows the proportion of the known population each year that were also known to be informed, and (C) shows the proportion of observed feeding events each year that were lobtail feeding, along with sand lance abundance in research trawls (27).



NBDA uses an association matrix, which estimates the proportion of time that individuals are associated, to quantify the extent to which social network structure explains the spread of a behavior (9, 19). The underlying assumption is that socially transmitted behaviors should spread at a higher rate between individuals who spend more time associated. We used both discrete time-of-acquisition diffusion analysis (TADA) and the order-of-acquisition extension (OADA) to compare the social association matrix with the order in which informed individuals acquired the trait (9, 22). NBDA models the spread of behavior as a stochastic process in which, at any given time, each naïve individual has a learning rate that determines the likelihood of its learning the behavior at that time. NBDA also allows individual-level data to be incorporated to control for the possibility that factors such as age and gender could affect social learning rates (22).

We analyzed data collated by the Whale Center of New England (WCNE) from observers placed aboard commercial whale-watching vessels. The data set encompasses 27 years (1980–2007) and 73,790 sighting records, in which 653 individuals were sighted 20 or more times in and around the Stellwagen Bank National Marine Sanctuary (Fig. 1A). Lobtail feeding was first recorded in this data set in 1981, although the very first record comes from a separate study in which it was noted once in a sample of 150 feeding events observed in 1980 (13). Over the study period, the proportion of the population known to be informed increased to 37%, indicating that knowledge of this behavior has spread over time (Fig. 1B). The actual use of the lobtail feeding technique, measured as the proportion of observed feeding events in a given year in which lobtail feeding was used, did not follow a monotonic trend, showing instead a pattern of peaks and

Table 1. Summed Akaike weights (ω_i) and maximum-likelihood estimates of social transmission parameters for NBDA models with and without social transmission.

Analysis type	Social transmission model	$\Sigma \omega_i$	Social transmission parameter estimate	95% CI
Order of acquisition (OADA)	Multiplicative	0.84	30.9	8.2–123.2
	Additive	0.16	32.0	11.3–96.6
	No social transmission	1.8×10^{-23}	(Constrained to 0)	—
Year of acquisition (discrete TADA)	Multiplicative	0.88	2.7	1.2–5.3
	Additive	0.12	3.0	1.1–8.5
	No social transmission	7.7×10^{-7}	(Constrained to 0)	—

troughs. The first observations of lobtail feeding were associated with a peak in sand lance abundance, and a subsequent sharp increase in the use of the behavior was closely tied to a second abundance peak in 1987–1989 (Fig. 1C).

Models including social transmission were overwhelmingly supported in both the OADA and TADA analyses, by between 6 and 23 orders of magnitude as compared to an equivalent set of models in which the social transmission effect was constrained to zero (Table 1). Estimates of the social transmission effect, the factor by which an individual's learning rate is increased per unit of association with informed individuals as compared to the asocial learning rate of an average individual in the population, ranged from 2.7 to 32.0, with no 95% confidence intervals (CIs) spanning zero. The OADA and TADA models respectively estimated that 87 and 45% of whales that acquired lobtail feeding did so by social transmission, with the latter estimate likely to be highly conservative (22). An alternative explanation for this pattern is that whales who acquired lobtail feeding were subsequently more likely to associate with one another, perhaps through shared attraction to sand lance shoals. When we allowed

for such homophily effects by disregarding all associations recorded after each individual acquired the behavior (22), the ΔAIC (Akaike Information Criterion) between models with and without social transmission was 21.8, corresponding to 54000:1 support for the model with social transmission. These results show that social transmission has had a major role in the spread of lobtail feeding.

The social network in the population was consistent with previous descriptions of humpback social structure (14, 23), being characterized by many relatively weak associations. The social network of individuals sighted at least 20 times showed a mean number of associates at any one time, also known as mean node strength, of 1.04 (range 0.02 to 4.14) measured by the half-weight association index (22, 24), and the average number of identified associates for the 653 individuals was 51 (range 1 to 210). Modeling the network as a system of springs (25) shows informed individuals (defined as those observed lobtail feeding at least once) concentrated at the well-connected center of the network (Fig. 2).

In the OADA analysis, the nonsocial characteristic with the largest effect on an individual's rate of learning lobtail feeding was the proportion

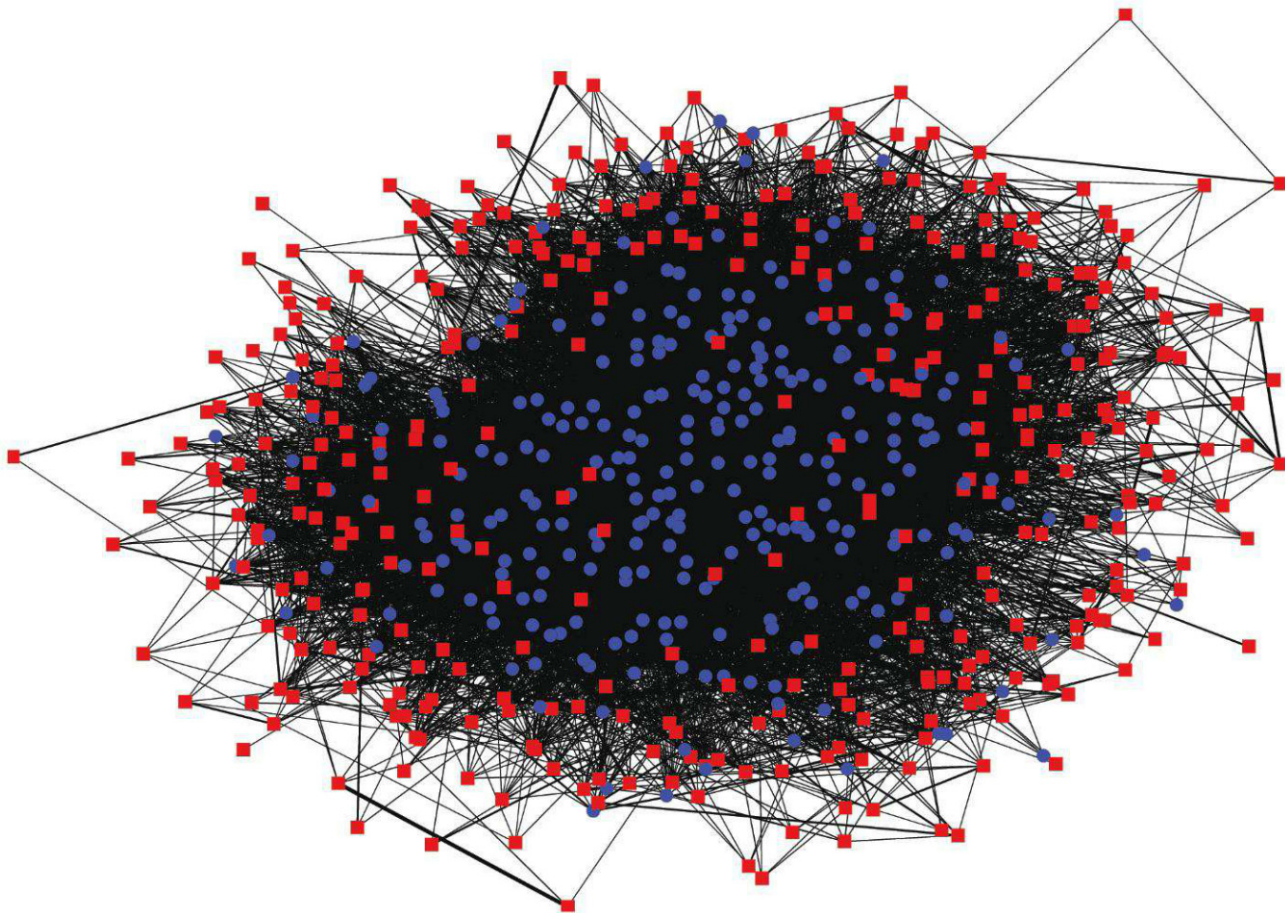


Fig. 2. Social network of whales sighted at least 20 times. Blue nodes are individuals observed lobtail feeding, red nodes are those never observed lobtail feeding. The network was laid out by spring-embedding using Netdraw (25) software.

Table 2. Individual-level effects. Summed Akaike weights (ω_i), model-averaged parameter estimates, and maximum-likelihood effect sizes for individual-level variables from multiplicative OADA models. Effect sizes are only interpreted where there is more support for a variable than against ($\omega_i > 0.5$).

Variable	$\Sigma \omega_i$	Estimate	95% CI	Effect size	Interpretation
Age	0.713	−0.109	−0.34–0.03	0.9827	Learning rate decreases by factor of 0.98 per year of age.
Number of sightings	0.626	0.058	−0.05–0.23	1.0003	Apparent learning rate increases by factor of 1.0003 per sighting (e.g., by 1.03 or 3% for median number of sightings).
Stellwagen residency	0.616	0.616	−0.37–2.48	1.8514	Individual seen exclusively on the bank has a learning rate increased by 80% as compared to one never seen on the bank.
Sex	0.460	−0.040	−0.25–0.09	0.9222	Little support for gender effect
Lobtail-feeding mother	0.353	−0.034	−0.37–0.14	0.9352	Little support; not consistent with major genetic influence

of times that individual was sighted within the Stellwagen Bank sanctuary area (Table 2). We interpret this as indicating a role for ecological factors in promoting the acquisition of the feeding technique, but the effect size (an 80% increase in learning rate for an individual sighted exclusively within the sanctuary) does not approach the estimated effect of social transmission. In the TADA analysis, we were able to include annual sand lance biomass estimates as a predictor of learning rates, and it received strong support [summed Akaike weights ($\Sigma \omega_i$) = 0.998], with a positive effect on asocial learning rates (a 40% increase per kilogram of increase in mean annual sand lance weight in research trawls, table S1), providing direct evidence of a link between lobtail feeding and sand lance. The OADA analysis indicated that having an informed mother has virtually no effect on learning rates (Table 2), whereas in the TADA analysis, having an informed mother was well supported as a predictor, but its effect was estimated as negative (table S1). Both of these results are inconsistent with a significant role for genetic factors or vertical social learning in the acquisition of lobtail feeding, showing that horizontal cultural transmission is largely responsible for the spread of the behavior. Most learning

of this behavior occurred after weaning—just 2% of the 241 informed individuals of known age were first seen lobtail feeding when less than 2 years old—and mothers do not preferentially associate with their offspring after weaning (14), which helps explain the lack of maternal influence.

A powerful advantage of the NBDA approach is that it allows the simultaneous consideration of ecological, social, and genetic factors as predictors of individual learning rates, thus moving away from sterile arguments about excluding such factors in the development of behavior and instead reflecting the reality that all behavior develops as an interaction of multiple factors (1). Thus, in the present study we are able to describe the roles of both ecology and social transmission in the spread of a feeding innovation, reflecting the notion that social learning allows dynamic adaptation to changing ecological circumstances. Lobtail feeding first appeared in this population during a rapid rise in the abundance of sand lance, which gather in high densities to spawn on Stellwagen Bank, after a crash in another important prey, herring (18). Although the purpose of adding a lobtail to the beginning of a bubble-feeding dive sequence is unknown, the link with contemporaneous prey dynamics suggests some function specific to foraging on sand lance, perhaps provoking a tightening of the prey school before bubble entrapment. Our results show that social transmission played a crucial role in the spread of lobtail feeding behavior, which has now persisted over 27 years and multiple generations (14). Lobtail feeding can therefore be considered a tradition (26), and because humpback populations are known to also carry vocal traditions in

the form of song (10, 11), this population can be considered to carry multiple traditions.

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Supplementary Materials

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Materials and Methods
Table S1
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Database S1

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Population Growth in a Wild Bird Is Buffered Against Phenological Mismatch

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Broad-scale environmental changes are altering patterns of natural selection in the wild, but few empirical studies have quantified the demographic cost of sustained directional selection in response to these changes. We tested whether population growth in a wild bird is negatively affected by climate change–induced phenological mismatch, using almost four decades of individual-level life-history data from a great tit population. In this population, warmer springs have generated a mismatch between the annual breeding time and the seasonal food peak, intensifying directional selection for earlier laying dates. Interannual variation in population mismatch has not, however, affected population growth. We demonstrated a mechanism contributing to this uncoupling, whereby fitness losses associated with mismatch are counteracted by fitness gains due to relaxed competition. These findings imply that natural populations may be able to tolerate considerable maladaptation driven by shifting climatic conditions without undergoing immediate declines.

When environments change over time, individuals with extreme trait values can have higher fitness in the new environment, and thus directional selection toward that end of the phenotype distribution will occur

(1). Given a heritable basis to trait variation, evolutionary responses may ensue (2), but a major concern is that the rate of environmental change might outstrip the pace of evolutionary adaptation, thereby threatening the persistence of popu-

lations and species (3, 4). Theoretical models have explored this problem by framing the issue in terms of fitness landscapes, where environmental change causes the fitness optimum to shift through trait space (4–8), rendering the population less well-adapted to its environment as compared to the situation before the change. As the lag between mean phenotype and optimum phenotype increases, the strength of directional selection toward the optimum intensifies, but the population is also expected to accrue an increasing “lag load,” or demographic cost, in terms of reduced mean fitness (5–10). Despite the central importance of these concepts in eco-evolutionary theory and conservation biology (7, 11, 12), empirical studies quantifying the demographic cost of selection in wild populations are lacking [but see (13)].

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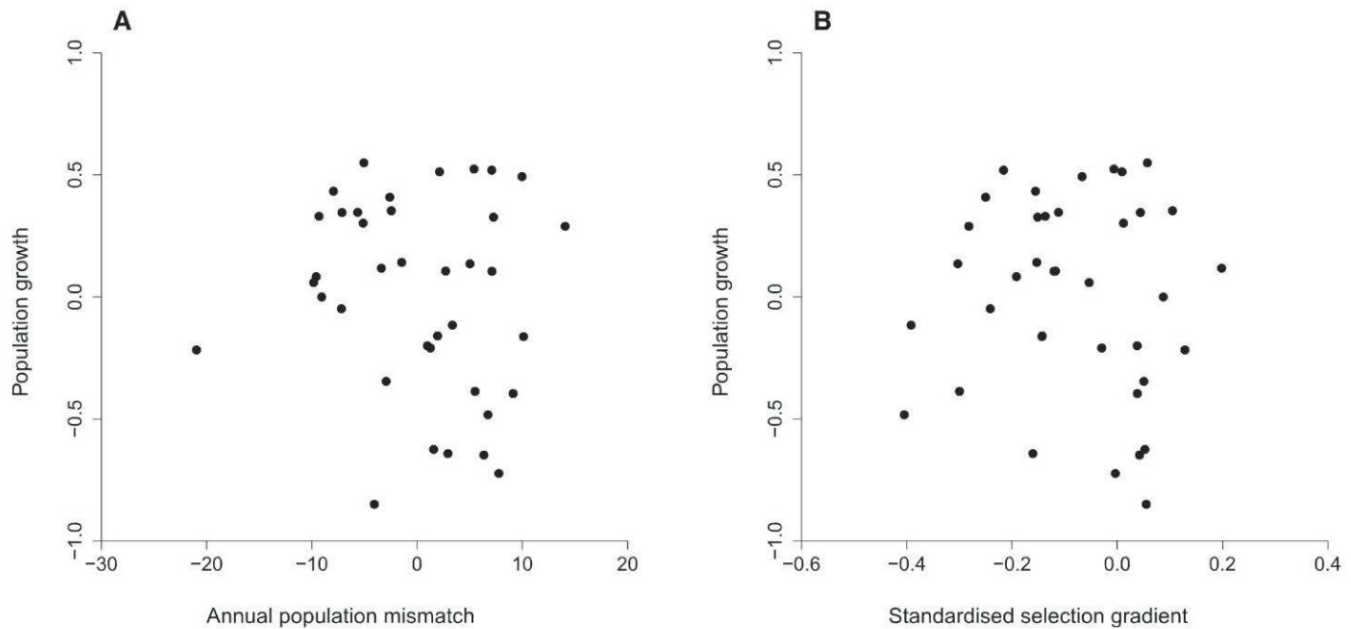


Fig. 1. Population growth as a function of (A) annual population mismatch and (B) annual standardized selection gradient. (A) Positive values on the y axis indicate population increase, negative values indicate population decrease. Positive values on the x axis in-

dicate years when mean breeding time is later than the food peak, negative values the opposite. (B) Positive selection gradients indicate selection for later laying dates, negative gradients indicate selection for earlier laying.

Table 1. Results of the GLMM on fitness contributions to population growth ($W_{i,\tau}$). All explanatory variables were mean- and variance-standardized before model fitting, hence effect sizes are directly comparable. None of the two-way interactions were significant ($P > 0.05$). A GLMM with Poisson errors and log-link function was used; estimates are on a log scale. Random effects were as follows: female identity variance component = <0.001 ; year variance component = 0.046. Number of observations = 4177; groups: female identity = 2616; year = 38.

	Estimate	Standard error	z value	Probability ($> z $)
<i>Significant fixed effects</i>				
Intercept	-0.615	0.044	-14.001	<0.001
Breeding density	-0.220	0.046	-4.738	<0.001
Beech crop	0.185	0.043	4.354	<0.001
Age structure	-0.094	0.044	-2.159	0.031
<i>Nonsignificant fixed effects</i>				
Population mismatch	-0.054	0.042	-1.260	0.208
Population mismatch ²	0.040	0.032	1.254	0.210
Beech crop ²	-0.032	0.054	-0.585	0.558

Climate change can disrupt preexisting synchrony between interacting species in the seasonal timing (i.e., phenology) of their life-history events (14). For example, phenological mismatches between predators and prey are expected to affect both the demography of predator populations (15) and selection pressures on traits affecting phenology (3), providing an ideal opportunity to characterize the demographic cost of selection. We studied great tits (*Parus major*) in the Netherlands in relation to the phenology of their caterpillar food supply. This part of Western Europe has experienced substantial spring warming in recent decades related to global climate change (16). Great tits rely on caterpillars to feed their chicks and strive to match their breeding time with the pronounced seasonal peak in caterpillar biomass,

which enhances offspring survival (16–19). Previous studies illustrated how climate change has produced a steadily increasing mismatch between great tit and caterpillar phenology in our study area, because the caterpillar food peak has advanced in response to rising spring temperatures at more than twice the rate of great tit laying dates (16, 17). When temperatures during the period after great tits have laid their eggs (late spring) are high, the mismatch is larger (by 2.96 ± 0.43 days per 1°C increase, $F_{1,36} = 47.40$, $P < 0.001$), because caterpillars develop faster under warmer conditions (19) and hence the food peak is early relative to the great tit nestling phase. The greater this mismatch, the stronger is directional selection for earlier laying dates (linear regression slope = -0.007 ± 0.003 , $F_{1,36} = 5.066$, $P = 0.031$).

If phenological mismatch has detrimental fitness consequences, we expect a negative relationship between annual population growth and population mismatch (*PM*); i.e., reduced population growth in years when breeding is late relative to the food peak. However, there were no statistically significant linear ($F_{1,36} = 0.277$, $P = 0.602$) nor quadratic ($F_{2,35} = 0.179$, $P = 0.602$) effects of *PM* on population growth (Fig. 1A). Moreover, there was no relationship between population growth and directional selection strength (Fig. 1B; linear regression: $F_{1,36} = 0.040$, $P = 0.842$; quadratic regression: $F_{2,35} = 0.956$, $P = 0.394$), implying that mistiming (the deviation between mean laying dates and the moving fitness optimum) did not depress mean fitness. To explore this apparent uncoupling between population growth and *PM* in more detail using individual-level demographic data, we defined a fitness variable $W_{i,\tau} = R_{i,\tau} + S_{i,\tau}$, where $R_{i,\tau}$ was the number of female recruits per breeding female per year and $S_{i,\tau}$ was a binary variable indicating survival to breed again the next year (20, 21). We then fitted a generalized linear mixed-effects model (GLMM) (21) with $W_{i,\tau}$ as the response variable and used it to test whether interannual variation in absolute fitness (which is strongly positively correlated with population growth, fig. S1) was associated with variation in *PM*. There were no statistically significant linear or quadratic effects of *PM*, even after controlling for the effects of interannual variation in beech nuts [a key winter food source (20)], breeding population size, and age structure (Table 1). This confirmed that between-year variation in phenological mismatch did not explain the observed

variation in annual mean fitness and thus did not influence population growth.

These results present a conundrum: Why is population growth not lower in years when a large fraction of females lays too late? There are two reasons. The first is simply that the food peak is typically much narrower than the distribution of laying dates, and hence reproductive fitness cannot be high for all females each year. In years when the mean laying date was early relative to the food peak (negative *PM* years), early females produced fewer fledglings (Fig. 2A) and fewer recruits (Fig. 2B) than late females, whereas intermediate females had the highest reproductive output. In years when mean laying date was relatively late (positive *PM* years), late females had lower reproductive output than intermediate females, who in turn performed more poorly than the earliest females (Fig. 2, A and B). Thus, the overall pattern of fecundity selection has shifted from stabilizing to directional as the population breeds increasingly late relative to the food peak, but average reproductive

output has not been depressed appreciably. The patterns for viability selection (Fig. 2C) are similar, except that in negative *PM* years the latest females have the highest survival, whereas in positive *PM* years they have the lowest survival, potentially because they pay higher energetic costs when provisioning their broods under conditions of food shortage (22).

The second reason is that reduced annual fledgling production associated with phenological mismatch (23) is counterbalanced by improved post-independence survival of offspring, due to relaxed competition. The survival of juveniles is strongly density-dependent in our study population (23, 24); consequently, the total number of new recruits to the breeding population each year is, in fact, unrelated to the number of fledglings produced the previous year (fig. S2). Fewer fledglings in years of strong mismatch, therefore, do not necessarily lead to fewer recruits overall (fig. S2), even though breeding time still determines which females produce the most recruits. Of the two explanations, this latter buffering

mechanism is the most general, because density-dependent compensation (DDC) is a widespread phenomenon in natural populations. For example, sustainable harvesting of wildlife and fish populations is typically based on the premise that increased mortality due to harvest will be partially or fully offset by decreased natural mortality (or increased fecundity) after harvest because of reduced competition for food or territories (25, 26). Our proposed mechanism is analogous, except that nestling mortality due to phenological mismatch (rather than harvest) reduces cohort size at the end of the breeding season, which in turn increases per-capita recruitment rates (fig. S2).

The first mechanism implies that if *PM* were to increase further, directional selection would continue to intensify and mean fitness would eventually decrease (in the absence of an evolutionary response). The second mechanism implies, however, that the reduction in mean fitness would be much stronger if not for DDC. To illustrate the interaction between the two mechanisms, we simulated the stochastic population dynamics using

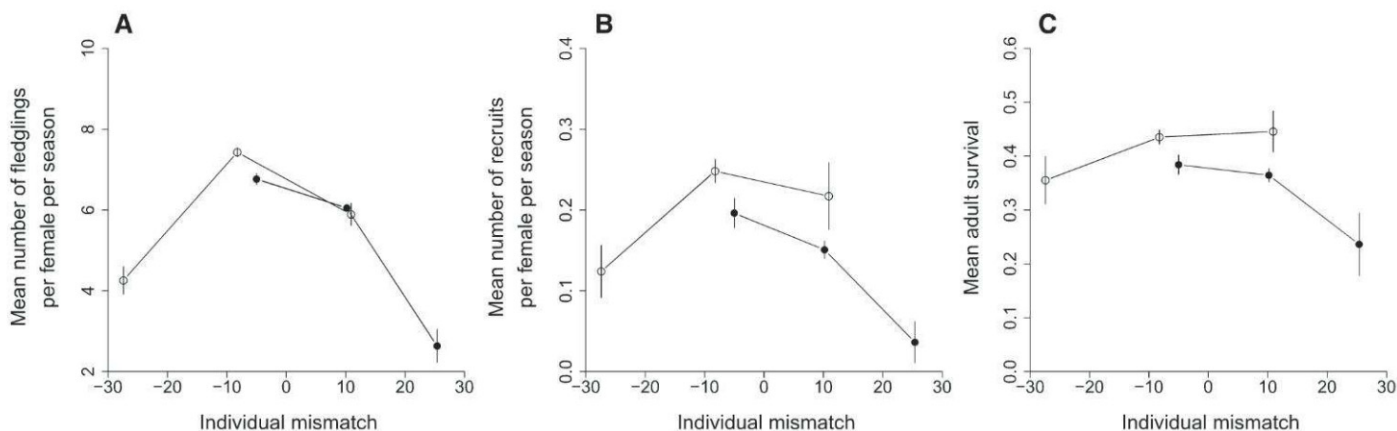
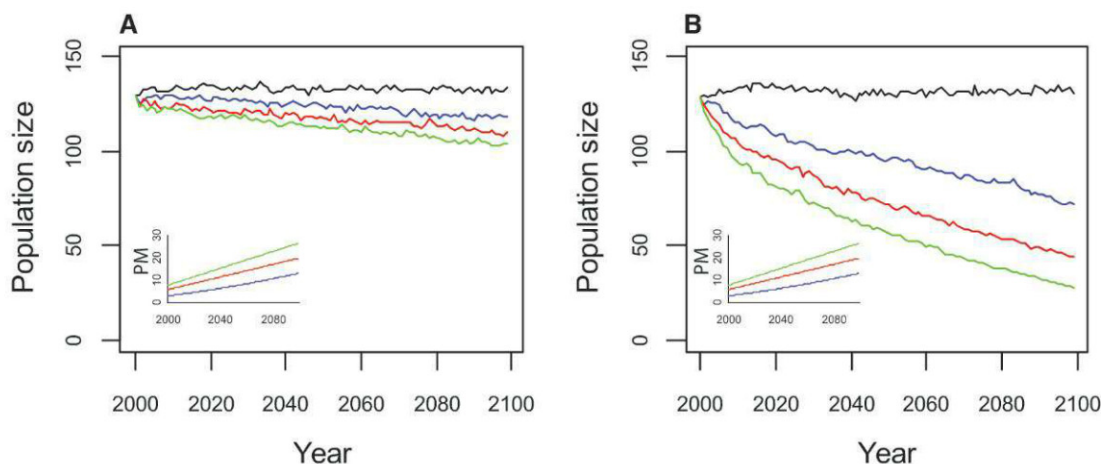


Fig. 2. Fitness components plotted against individual mismatch. (A) Mean number of fledglings, (B) mean number of female recruits, and (C) mean adult female survival per year plotted against individual mismatch. For illustrative purposes, data were grouped into negative mismatch years ($n = 17$ years, open

circles), when the mean breeding time was earlier than the food peak, and positive mismatch years ($n = 21$ years, solid circles), when mean breeding time was later. Data were also binned along the x axis into three equally spaced categories, corresponding to early, intermediate, and late breeders. Error bars are SEM.

Fig. 3. Simulation model results when recruitment was (A) density-dependent and (B) density-independent. Results of the simulation model, in which stochastic population dynamics were projected under a control scenario of no climate change (black = no temporal trend in *PM*) and three scenarios of climate change (blue = mild, red = moderate, and green = strong).

In (A), the observed relationship between recruitment and breeding density was used, whereas in (B) the slope of this relationship was set to zero (i.e., density dependence was “turned off”). Shown are median population sizes across 1000 simulations. The small inset graphs show inputted trends in *PM* corresponding to each climate change scenario.



the observed relationships between recruitment/survival and mismatch (21), and projected the population forward in time under the same three scenarios of climate change as described in (27). For each scenario, predicted changes in *PM* were first calculated using output from models of great tit and caterpillar phenology described in (27) and then used as inputs to the current simulation model. The simulations showed that a gradual decline in population size would result if *PM* increases over the next century (assuming no evolutionary response and the same DDC in recruitment rates as observed in the historical data), with the decline being strongest under the most extreme scenario of climate change (Fig. 3A). When DDC in recruitment rates was “turned off” in the simulations, the rate of population decline for each scenario became substantially stronger (Fig. 3B). This demonstrates that DDC modulates the impacts of mismatch on population growth by partially buffering the population from otherwise more rapid declines.

We found that increasing phenological mismatch driven by climate change had little effect on population growth rates over a study period of almost four decades, despite intensifying directional selection on laying dates. Population numbers therefore remained stable even though late spring temperatures increased by 3.7°C (21). Density dependence within the life cycle partially buffered population growth rate against the negative effects of environmental change, in effect dampening the demographic cost of directional selection. Indeed, theory suggests that the magnitude of the lag load will depend on the form and strength of density regulation, as well as the magnitude of stochastic environmental fluctuations (28). The DDC mechanism we describe here is therefore likely to be of general importance in a wide range of species. For example, Wilson and Arcese (29) found that the population growth of song sparrows was not affected by year-to-year variation in the timing of breeding, despite the latter being strongly correlated with climate. They suggested, but did not demonstrate explicitly, that DDC in recruitment rates could explain this uncoupling. Similar buffering mechanisms might also play a role in dampening the effects of climate change on density-regulated mammal populations, although reductions in mean fitness may be unavoidable when large phenological changes occur (30). Further studies characterizing the lag load in wild populations with different life histories will be crucial to understanding and predicting climate change impacts on population dynamics. Evolutionary adaptation will be critical for persistence in the long run (27), but our results imply that considerable directional selection might be demographically tolerable on decadal time scales without immediate population declines, effectively buying time for microevolution to restore adaptation.

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Supplementary Materials

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Figs. S1 and S2
References (31–33)

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Simultaneous Femtosecond X-ray Spectroscopy and Diffraction of Photosystem II at Room Temperature

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Intense femtosecond x-ray pulses produced at the Linac Coherent Light Source (LCLS) were used for simultaneous x-ray diffraction (XRD) and x-ray emission spectroscopy (XES) of microcrystals of photosystem II (PS II) at room temperature. This method probes the overall protein structure and the electronic structure of the Mn₄CaO₅ cluster in the oxygen-evolving complex of PS II. XRD data are presented from both the dark state (S₁) and the first illuminated state (S₂) of PS II. Our simultaneous XRD-XES study shows that the PS II crystals are intact during our measurements at the LCLS, not only with respect to the structure of PS II, but also with regard to the electronic structure of the highly radiation-sensitive Mn₄CaO₅ cluster, opening new directions for future dynamics studies.

One of the metalloenzymes most critical for sustaining aerobic life is photosystem II (PS II)—a membrane-bound pro-

tein complex found in green plants, algae, and cyanobacteria—that catalyzes the light-driven water oxidation reaction. The oxidation equivalents

generated by the absorption of four photons by the PS II reaction center are stored in the four consecutive redox states of a Mn_4CaO_5 cluster, known as the S_i ($i = 1$ to 4) states. The accumulated energy is used in the concerted oxidation of two molecules of water to form dioxygen (O_2), returning the catalyst to the most reduced S_0 state in the Kok cycle (Scheme 1). Due to its efficient catalysis of the demanding four-electron and four-proton chemistry of water oxidation, the Mn_4CaO_5 cluster has been a model system for synthesizing inorganic water oxidation catalysts (2, 3).

The structure of PS II in its dark stable state (S_1) was studied extensively using x-ray diffraction (XRD) measurements on cryo-cooled crystals (4–7) at synchrotron radiation (SR) sources with resolutions ranging from 3.8 to 1.9 Å. One inherent limitation of XRD measurements on this system, however, is the high radiation sensitivity of the Mn_4CaO_5 cluster. An increase of the average metal-ligand and metal-metal distances is observed in the XRD data as compared with the extended x-ray absorption fine structure (EXAFS) data that were collected below the threshold of radiation damage [reviewed in (8)], indicating that the structure of the cluster is either altered or disrupted. Such specific damage (photoreduction of the metal center) (9, 10) occurred despite the fact that all XRD measurements were carried out at cryogenic temperatures of 100 to 150 K. It is now generally recognized that for some other redox-active metalloproteins, it is difficult to obtain intact structures with SR-based XRD, even at cryogenic temperatures (11, 12). Recently, a new approach to protein crystallography was demonstrated at the X-ray Free-Electron Laser (XFEL) of the Linac Coherent Light Source (LCLS), with ultrashort x-ray pulses of high intensity enabling collection of diffraction data at room temperature (RT) before the onset of radiation damage in various systems (13–18).

Whereas XRD is powerful in determining the overall protein structure, various x-ray spectroscopy techniques can provide critical complementary information about the active site due to their element and chemical sensitivity (8, 19–22).

To understand the intricate interplay between protein and metal cofactors that allows complex reactions, it is desirable to combine both approaches in time-resolved studies under functional conditions.

Among the various spectroscopic methods, nonresonant x-ray emission spectroscopy (XES) probes occupied electron levels (see Fig. 1, bottom right). In particular, the $\text{K}\beta_{1,3}$ line is a probe of the number of unpaired 3d electrons, hence providing information about the oxidation and/or spin state (22). Experimentally, XES performed using an energy-dispersive x-ray spectrometer (23) is particularly well suited for such combined shot-by-shot studies, as excitation energies above the 1s core hole of first-row transition metals are also ideal for XRD; therefore, neither incident nor emitted photon energy need to be scanned. Although it has been demonstrated that the shot-by-shot approach can probe the atomic structure of intact proteins at high resolution (17), the question has remained whether ultrabright femtosecond pulses can also probe the intact electronic structure of active centers such as the

Mn_4CaO_5 cluster. This is by no means obvious, because, in contrast to XRD, in which radiation-induced damage leads to loss of diffractivity, such a self-termination of the signal is not expected in XES, and electronic structural changes happen on a much faster time scale than a Coulomb explosion. Recently, we used solutions of Mn model systems (24) in a liquid jet (25) at LCLS to demonstrate the feasibility of RT femtosecond $\text{K}\beta$ XES.

Building on the feasibility of these separate femtosecond XRD studies of PS II (18) and femtosecond XES results, we have designed an experimental setup for simultaneous XRD and XES data collection at the LCLS. We used XES to determine the electronic-state integrity of the Mn_4CaO_5 cluster. Simultaneously, we used a visible-laser pump (centered at 527 nm) and an x-ray laser probe to collect RT XRD measurements of the S_2 state, and we compared our results with the XRD data from the dark S_1 state. The schematic of the setup is shown in Fig. 1 (26). Suspensions of PS II microcrystals (5 to 15 μm in the longest dimension) were

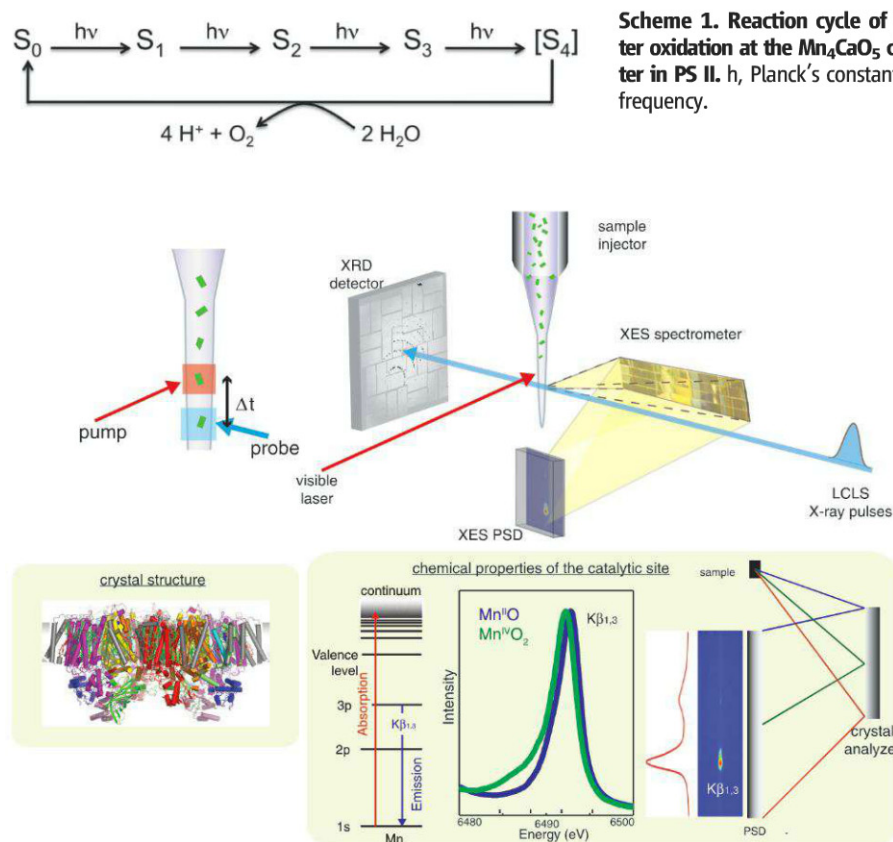


Fig. 1. Setup of simultaneous x-ray spectroscopy and crystallography experiment at the CXI instrument of LCLS. The crystal suspension is electric-field-focused into a microjet that intersects the x-ray pulses. XRD data from a single crystal are collected downstream with a CSPAD detector, and XES data from the same crystal are collected at $\sim 90^\circ$ to the beam via a multicrystal XES spectrometer and a compact position-sensitive detector (PSD). A Nd:YLF laser (527 nm) is used to illuminate the crystals. The timing protocol (top left) consists of a fixed time of flight Δt between the optical pump and x-ray probe. The schematic of the energy dispersive spectrometer is shown (bottom right), as well as the Mn^{II} and Mn^{IV} oxide $\text{K}\beta_{1,3}$ spectra and an energy-level diagram for XES (bottom middle).

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injected into the coherent x-ray imaging (CXI) chamber (27) at LCLS using an electrospun liquid microjet (25) to intersect the x-ray pulses (~50-fs pulse length, peak x-ray dose of ~150 megagray per pulse). We used a Cornell-SLAC pixel array detector (CSPAD) (28) to collect XRD data. Mn $K\beta_{1,3}$ XES data were collected simultaneously from the same sample using an energy-dispersive spectrometer at ~90° to the beam direction and a small CSPAD (26).

The dark-adapted PS II microcrystals showed diffraction spots to 4.1 Å in the best cases (fig. S1). Within 5 hours of run time (corresponding to nearly 2,200,000 x-ray shots), we collected 90,000 shots identified as potential hits (16 or

more strong Bragg peaks). Out of these, 4663 shots were indexed and integrated (tables S1 and S2). We processed the diffraction data to a resolution of 5.7 Å to generate the electron density map shown in Fig. 2, A and B [the 5.7 Å cutoff was chosen on the basis of the multiplicity and completeness (tables S1 and S2)]. However, we indexed Bragg spots to 4.1 Å resolution, and the signal strength, as measured by $I/\sigma(I)$ (the ratio of the spot's peak intensity to its standard deviation), was still 1.9 out to 5 Å resolution (table S2).

The improved resolution of the electron density compared with that reported earlier at 6.5 Å resolution (18) is manifested in a more detailed

map allowing for better tracing of the transmembrane helices and of the loop regions in the membrane extrinsic areas of the complex facing the inner compartment of the thylakoid (lumen) and the cytoplasm (stroma). There is good agreement between the 5.7 Å resolution electron density from the XFEL data (Fig. 2A) and previously collected (6) SR data [Protein Data Bank identification number (PDB ID) 3bz1] truncated at 5.7 Å. Despite slight nonisomorphism between the XFEL and SR data sets, the maps have an overall correlation coefficient (CC) of 0.36 (a CC of 0 means no correlation; a CC of 1 indicates full correlation).

The influence of the phasing model on the electron density omit maps was tested by excluding heavy elements (see supplementary materials and fig. S2). The use of random or uniform structure factors instead of the experimental data did not generate any density peaks in the omit maps in the region of the Mn_4CaO_5 cluster, confirming that the density observed is from the experimental data (fig. S3). It is similar to the density obtained from the SR data but is slightly more compact (Fig. 2B).

Figure 3A shows the $K\beta_{1,3}$ x-ray emission spectra from PS II crystals collected simultaneously with the XRD data. The spectra average ~20,000 shots, recorded at 7-keV incident energy, that we identified as crystal hits from the XRD data. To illustrate the sensitivity of this measurement, we estimated that the volume of sample probed by x-rays for this spectrum is ~0.3 nL, containing a total of 2×10^{-12} mol Mn. A spectrum using only data from crystals that yield indexable diffraction patterns was computed as well (fig. S4). Both spectra coincide in peak position and shape and differ only in the signal-to-noise (S/N) ratio due to the different number of samples used for each of them. The spectrum of PS II crystals matches very well with the spectrum from dark-adapted PS II solution (S_1 state) (8.9 mM Chl, 1 mM Mn, 375,000 individual shots) collected with the same setup at the CXI instrument (Fig. 3B). This implies that the Mn cluster is in the same high-valent state ($Mn_2^{III}Mn_2^{IV}$) in both micrometer-sized crystals and solution and that the crystallization procedure did not alter the native PS II S_1 state.

We used the SR solution data collected at 8 K and at RT to compare the shape and energy position of the spectra. The RT SR spectrum represents a completely photoreduced (damaged) PS II (Fig. 3C, pink), where all Mn is reduced to Mn^{II} and the $K\beta_{1,3}$ peak is shifted toward the position found for $Mn^{II}Cl_2$ in aqueous solution (Fig. 3C, gray). The 8-K SR spectrum is from an intact PS II S_1 state (Fig. 3C, light blue). It is evident that the XFEL PS II XES data at RT are identical to the intact S_1 state spectrum. Note that we used roughly the same x-ray dose for the XFEL and the SR measurements at RT (total number of photons per sample spot for the SR data and the number of photons per shot for the XFEL data). The result clearly demonstrates

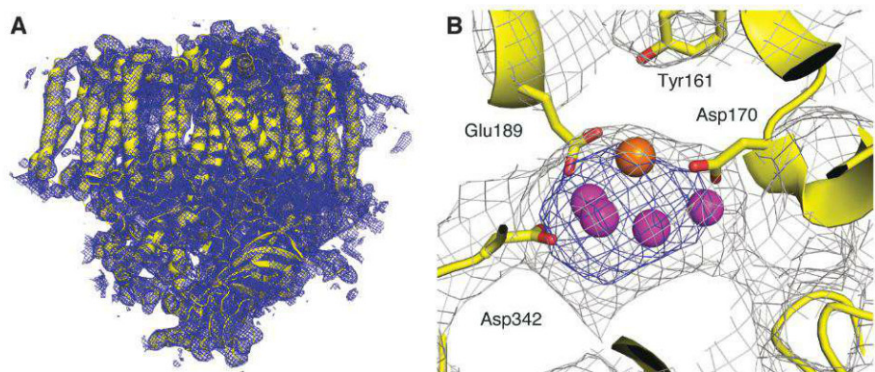


Fig. 2. Structure deduced from diffraction of micrometer-sized crystals of PS II using sub-50-fs x-ray pulses at RT at LCLS. (A) $2mF_o - DF_c$ electron density map for the PS II complex in the dark S_1 state obtained at LCLS. One monomer of the protein is shown in yellow, and the electron density is contoured at 1.2σ (blue mesh; shown for a radius of 5 Å around the protein). (B) Detail of the same map in the area of the Mn_4CaO_5 cluster in the dark S_1 state, with mesh contoured at 1.0σ (gray) and 4.0σ (blue). Selected residues from subunit D1 are labeled for orientation; Mn is shown as purple spheres and Ca as an orange sphere (metal positions taken from PDB ID 3bz1).

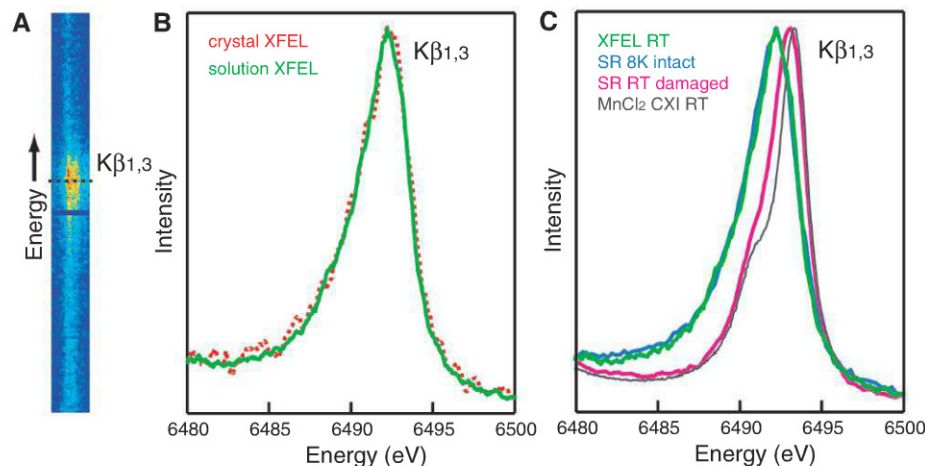


Fig. 3. Femtosecond XES of PS II. (A) Two-dimensional (2D) $K\beta_{1,3}$ x-ray emission spectra from microcrystals of PS II collected with a PSD at the CXI instrument using sub-50-fs pulses of about 2 to 3×10^{11} photons per μm^2 and pulse. (B) X-ray emission spectra of a solution of PS II (green curve) and single crystals of PS II (red dashed curve) in the dark state, both collected at the CXI instrument, obtained from the 2D plot in (A) by integration along the horizontal axis. (C) X-ray emission spectra of PS II solutions in the dark state collected at the CXI instrument at RT (green) or collected using SR under cryogenic conditions with a low x-ray dose ("8K intact," light blue) or SR at RT under photoreducing conditions ("RT damaged," pink). The spectrum from $Mn^{II}Cl_2$ in aqueous solution collected at RT at the CXI instrument is shown (gray) for comparison.

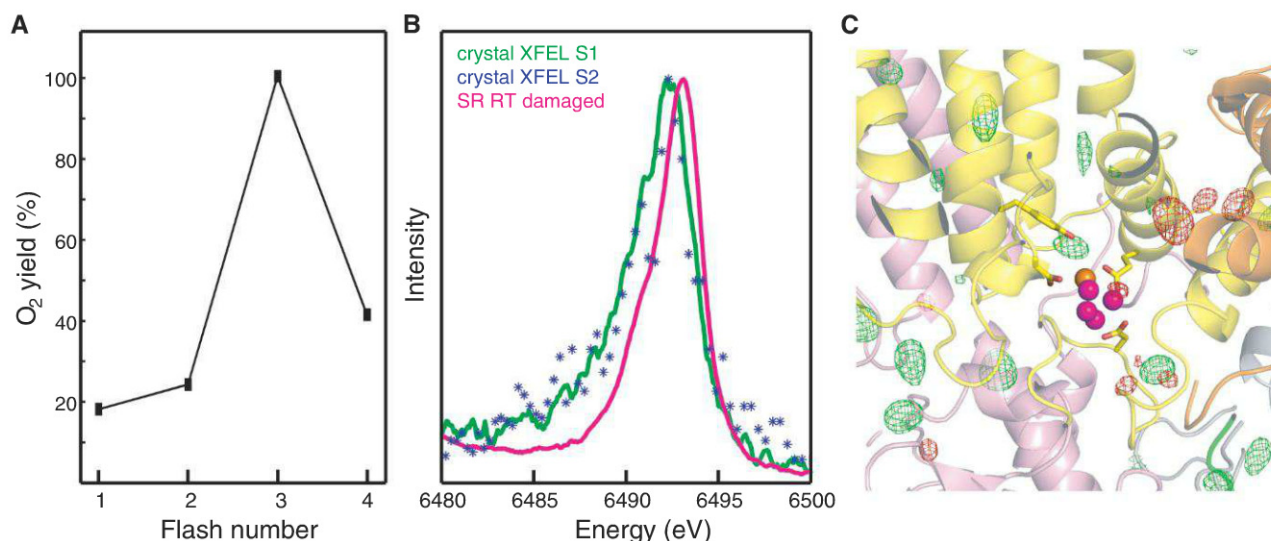


Fig. 4. Characterization of the illuminated PS II sample. (A) On-line MIMS measurements of light-induced O₂ yield detected as mixed labeled ¹⁶O¹⁸O species after illumination of photosystem II from *Thermosynechococcus elongatus*. The data shows that >73% of the sample occupies the S₂ state after one illumination. (B) XFEL XES of PS II in the S₂ state. The Kβ_{1,3} XES data collected from 362 microcrystals of PS II in the first illuminated S₂ state are shown in blue (asterisks). The XFEL spectrum of microcrystals of PS II in the dark stable S₁ state is shown in green. For comparison, an x-ray emission

spectrum of completely photoreduced ("damaged") PS II collected at RT at a synchrotron is shown in pink. (C) Isomorphous difference map between the XFEL-illuminated (S₂ state) and XFEL-dark (S₁ state) XRD data set in the region of the Mn₄CaO₅ cluster, with F_o-F_c difference contours shown at +3σ (green mesh) and -3σ (red mesh); histogram analysis indicates that this map is statistically featureless (fig. S6). Metal ions of the Mn₄CaO₅ cluster are shown for orientation as purple (Mn) and orange (Ca) spheres; subunits are indicated in yellow (D1), orange (D2), pink (CP43), and green (PsbO).

that the femtosecond x-ray pulses, under the conditions presented here, can be used to obtain the intact x-ray emission spectrum of the highly reduction-sensitive, damage-prone Mn₄CaO₅ cluster of PS II at RT. The XES data prove that the XRD data and the electron density are from PS II with a fully intact Mn₄CaO₅ cluster in the S₁ state (Mn^{III}Mn^{IV}) under the conditions of the current experiment.

The confirmation of an intact Mn₄CaO₅ cluster in PS II at RT allowed us to explore the illuminated state (S₂ state) with a visible-laser pump followed by an x-ray laser probe pulse. The crystals were illuminated in situ using the 527-nm output of a Nd–yttrium–lithium–fluoride (Nd-YLF) laser with a delay time between optical and x-ray exposure of 0.4 to 0.5 s (26). Advancement of the PS II sample into the S₂ state was tested independently with a similar illumination setup coupled to a membrane inlet mass spectrometer (MIMS) using water labeled with oxygen-18. Analysis of the labeled O₂ produced as a function of the number of laser flashes showed that an S₂ state population of ~80% was achieved with one flash (Fig. 4A and supplementary materials).

Using the same illumination conditions for PS II microcrystals at the CXI instrument, we obtained ~4300 indexed diffraction patterns within a collection time of 53 min (corresponding to ~380,000 shots). Out of these, 1850 diffraction images were included in the data set (XFEL-illuminated) with a final resolution of 5.9 Å. The statistics for both XFEL-dark and XFEL-illuminated data sets are given in tables S1 to S3. In parallel with the XRD, we also collected

XES data for these crystals. The resulting spectrum from 362 illuminated microcrystals recorded with an incident energy of 7 keV is shown in Fig. 4B (XFEL S₂). Despite the lower S/N ratio of the S₂ state spectrum, because of the fewer number of crystals sampled (a factor of ~50), the spectrum matches well with the x-ray emission spectrum obtained for crystals in the S₁ state. Detection of the expected shift (~60 meV) in the x-ray emission spectrum between the S₁ and S₂ states (20) requires a much better S/N ratio. However, it is evident that the XFEL S₂ state spectrum is different from the damaged spectrum, which clearly demonstrates that the Mn₄CaO₅ cluster is not photoreduced by our optical illumination pump protocol or the x-ray pulse.

The RT electron density maps of the dark and illuminated states are similar within the error of the resolution, with an overall CC of 0.77. An isomorphous difference map computed between the dark and illuminated data set showed no statistically significant peaks (Fig. 4C and figs. S5 and S6), and closer inspection of the region of the Mn₄CaO₅ cluster (Fig. 4C) and the stromal electron-acceptor side of the complex (fig. S5B) did not reveal any interpretable features in these regions. This shows that our illumination conditions do not lead to decay or changes in the crystal quality. More importantly, this finding suggests that there are no large structural changes taking place between the S₁ and S₂ states. Although the S₁-S₂ transition is accompanied by a number of changes in carboxylate and backbone vibration frequencies, as detected by infrared spectroscopy (29, 30), the

associated structural changes are most likely too small to be detected by the resolution achieved in the present study.

In summary, we have established that simultaneous XRD and XES studies using ultrashort, ultrabright x-ray pulses at LCLS can probe the intact atomic structure of PS II microcrystals and the intact electronic structure of its Mn₄CaO₅ cluster at RT. This technique can be used for future time-resolved studies of light-driven structural changes within proteins and cofactors and of chemical dynamics at the catalytic metal center under functional conditions. We expect that this method will be applicable to many metalloenzymes, including those that are known to be very sensitive to x-ray photoreduction and radiation damage, and over a wide range of time scales, starting with femtoseconds.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1234273/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S6
Tables S1 to S3
References (31–41)

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Insect Morphological Diversification Through the Modification of Wing Serial Homologs

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Fossil insects living some 300 million years ago show winglike pads on all thoracic and abdominal segments, which suggests their serial homology. It remains unclear whether winglike structures in nonwinged segments have been lost or modified through evolution. Here, we identified a ventral lateral part of the body wall on the first thoracic segment, the hypomeron, and pupal dorsolateral denticular outgrowths as wing serial homologs in the mealworm beetle *Tenebrio molitor*. Both domains transform into winglike structures under *Hox* RNA interference conditions. Gene expression and functional analyses revealed central roles for the key wing selector genes, *vestigial* and *scalloped*, in the hypomeron and the denticular outgrowth formation. We propose that modification, rather than loss, of dorsal appendages has provided an additional diversifying mechanism of insect body plan.

Insect ventral appendages, such as antennae, mouthparts, legs, and cerci, are serially homologous; they share anatomical and developmental features (1), despite morphological and functional differences along the body axis. The only known dorsal appendages in modern insects, however, are wings and their derivatives on the second and third thoracic segments. Winglike structures might have originally appeared on all thoracic and abdominal segments, and subsequently been repressed by *Hox* genes, which specify segment identities, in all but two thoracic segments (2). In the red flour beetle (*Tribolium castaneum*), RNA interference (RNAi) of the *Hox* gene *Sex combs reduced* (*Scr*) ortholog induced ectopic elytra formation in the pro-

thorax (T1, the first thoracic segment) (3). Furthermore, ectopic pairs of wings formed in abdominal segments when the *Hox* genes *Ultra-bithorax* (*Ubx*) and *abdominal-A* (*abd-A*) were repressed simultaneously (3). To explore putative wing serial homologs (WSHs), we examined the transformation from nonwing structure to wing by comparing weakly and strongly affected *Hox* RNAi phenotypes in the mealworm beetle (*Tenebrio molitor*), which is better suited to morphological analysis because it has a larger body than *T. castaneum*.

We initially isolated the *T. molitor* *Scr* homolog (*Tm-Scr*), and repressed its function by injecting double-stranded (ds) RNA into late-stage larvae (Fig. 1A). As in *T. castaneum*, ectopic elytra-like tissues formed on the first thoracic segment (T1) after *Tm-Scr* RNAi treatment (Fig. 1C), whereas dsRNA of the gene for enhanced green fluorescent protein (*egfp*) had no effect on adult phenotype (Fig. 1B). To identify the part that

transformed into elytra-like tissue, we compared the phenotypes of *egfp* dsRNA-treated T1 with mildly and severely affected *Tm-Scr* RNAi T1. The *T. molitor* prothoracic tergum (pronotum) extends to the ventral side and forms the posterior collar of the procoxae (4). This ventral region of the pronotum is termed the hypomeron (Fig. 1E, left). The severe phenotype showed almost complete transformation (Fig. 1, H, K, and N versus Fig. 1, F, I, and L), whereas the mild phenotype showed incomplete transformation (Fig. 1, G, J, and M). The latter may represent an intermediate morphology between nonwing structure and wing. The mild phenotype showed cleft formation on the dorsolateral sides (arrowheads in Fig. 1, G and M), and morphological changes in the lateral body wall from the cleft to the ventral side, which corresponds with the hypomeron. The hypomeron elongated to form ectopic elytra-like structures in severely affected T1. From this observation, we conclude that *Tm-Scr* RNAi induced transformation from hypomeron into the wing, which suggests that the hypomeron is a WSH in the T1.

Larval RNAi of *T. molitor* *Ubx* and *abd-A* (*Tm-Ubx* and *Tm-abd-A*) resulted in the formation of six additional pairs of wings on the first to sixth abdominal segments (Fig. 1D). We could not determine whether the ectopic wing is mesothoracic elytra-like or metathoracic membranous wing-like, because vein formation is insufficient, and severely affected adults die before complete sclerotization. We investigated which abdominal structures transformed into ectopic wings when both *T. molitor* *Ubx* and *abd-A* homologs (*Tm-Ubx* + *abd-A*) were knocked down. *T. molitor* pupae have paired denticular outgrowths of the body wall on the dorsolateral side of the first to seventh abdominal segments (Fig. 1E, right, and R). The jaws on the anterior and posterior sides of the projecting flange are strongly sclerotized and toothed to form the gin-trap, a protective

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device between segments (5, 6). It has been suggested that gin-traps grasp the appendages of attacking predators, such as mites and ants, in response to mechanical stimulation (5, 7, 8). The mildly affected *Tm-Ubx* + *abd-A* RNAi pupae had larger flanges than the *egfp* dsRNA-injected pupa, and the sclerotized jaws of the flange were absent (Fig. 1, P and S versus O and R, and fig. S1). In the severe RNAi phenotype, the dorso-lateral projections were elongated and formed ectopic wings (Fig. 1, Q and T, and fig. S1). These data suggest that the denticular outgrowth trans-

formed into the wing, and it therefore appears to be a WSH.

In *Drosophila melanogaster*, the metathoracic haltere is a serial homolog of the mesothoracic wing, as illustrated by the *bithorax* mutant, which bears metathoracic wings instead of halteres (9). In *Ubx* mutant clones, haltere tissue was transformed into wing tissue (10). This suggested that haltere cells have the potential to form wing tissue and that *Ubx* modifies their developmental context. The current study found that the prothoracic hypomerion and abdominal denticular

outgrowths have the developmental potential to form wings under *Hox* RNAi conditions, which suggests serial homology among the nonwing structures and the wing.

To verify this theory, we compared the development of the hypomerion, wing, and the denticular outgrowth. All of these structures grew dynamically during the late larval-to-prepupal stage and differentiated from the lateral portion of the larval epidermis, which was compartmentalized by muscle across the dorsoventral axis (fig. S2). The temporal and positional sim-

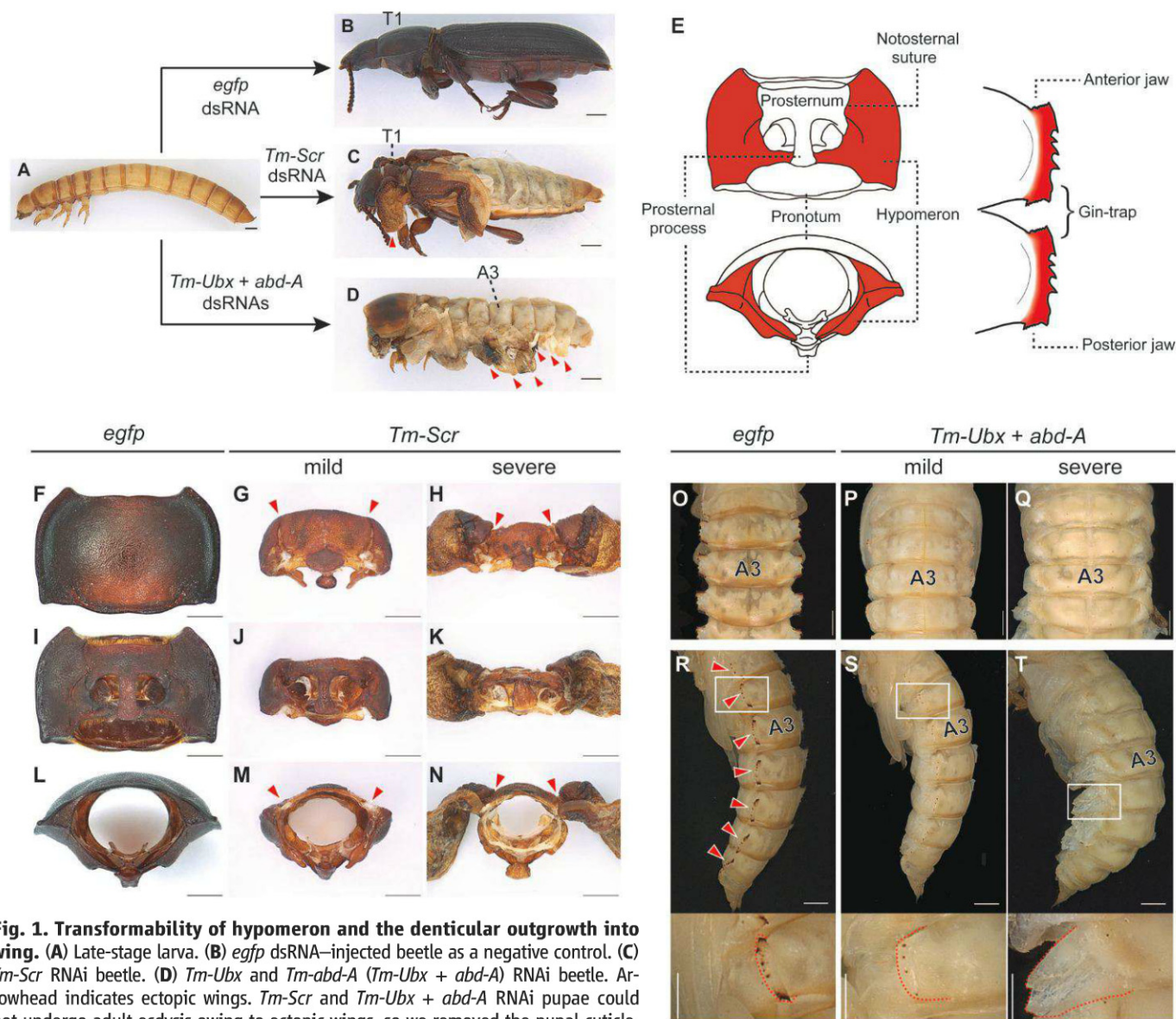


Fig. 1. Transformability of hypomerion and the denticular outgrowth into wing. (A) Late-stage larva. (B) *egfp* dsRNA-injected beetle as a negative control. (C) *Tm-Scr* RNAi beetle. (D) *Tm-Ubx* and *Tm-abd-A* (*Tm-Ubx* + *abd-A*) RNAi beetle. Arrowhead indicates ectopic wings. *Tm-Scr* and *Tm-Ubx* + *abd-A* RNAi pupae could not undergo adult ecdysis owing to ectopic wings, so we removed the pupal cuticle. (E) Schematic representation of hypomerion and the denticular outgrowth. (Left) The T1 without internal tissue and legs shown in ventral (top) and caudal (bottom) views. Hypomerion is shown in red. (Right) Dorsal view of the denticular outgrowths (red) in two abdominal segments. The gin-trap consists of a pair of jaws and a pit between segments. (F to N) Comparison of adult T1 phenotypes between *egfp* (F, I, and L) and *Tm-Scr* (G, H, J, K, M, and N) dsRNA-injected beetles shown in dorsal (F to H), ventral (I to K), and caudal (L to N) views. Arrowheads in (G), (H), (M), and (N) show the cleft formed at the dorsolateral side of the *Tm-Scr* T1. (O to T) Comparison of pupal phenotypes between *egfp* (O and R) and *Tm-Ubx* + *abd-A* (P, Q, S, and T) shown in dorsal (O to Q) and lateral (R to T) views. Arrowheads in (R) indicate the denticular outgrowths. The boxed region in (R) to (T) is enlarged below with the dorsolateral outgrowth indicated with dotted line. Second to sixth abdominal segments show similar phenotypes; therefore, those segments in comparable aspect are magnified. Mildly (P and S) and severely (Q and T) affected pupae are shown. A3, the third abdominal segment. Scale bar, 1 mm.

ilarities among hypomeron, wing, and the denticular outgrowth development suggest serial homology for these structures. Indeed, both of those lateral tissues in T1 and abdominal segment transformed into wing when we knocked down *Hox* genes (fig. S3), which supported this idea.

In *D. melanogaster*, Vestigial (*Vg*) physically interacts with Scalloped (*Sd*) to form a transcription factor complex that confers wing-field identity (11–13). Artificial expression of *vg* can induce ectopic winglike-structure formation in eyes, antennae, and legs (14). *vg* function in body-wall formation, other than in the wing, has not been reported. This specific and pivotal wing-formation function suggests that *vg* and *sd* are appropriate molecular markers of the wing field. To investigate the mRNA expression of *vg* and *sd* homologs (*Tm-vg* and *Tm-sd*) in nonwing segments, we performed in situ hybridization in T1, mesothorax (T2), and second abdominal segment (A2) transverse cryosections of middle-stage prepupae, in

which wing primordia undergo dynamic growth (Fig. 2A and fig. S2, F, K, and P). Localized expression of *Tm-vg* mRNA was detected at the distal tip of the developing mesothoracic wing (elytron) (Fig. 2, C and F, versus I). Specific expression of *Tm-vg* mRNA was also found in the developing hypomeron and the denticular outgrowth primordia at the same developmental stage (Fig. 2, B, D, E, and G, versus H and J). *Tm-sd* and *Tm-vg* mRNAs were clearly coexpressed, although the former showed a broader distribution (Fig. 2, K to P, versus Q to S). This was expected, because a pleiotropic function of *Sd* in concert with other cofactors [for example, growth regulation with Yorkie (15–17)] has been reported, whereas *vg* functions specifically in fly wing development.

We performed larval RNAi analysis to elucidate the functions of *Tm-vg* and *Tm-sd* in nonwinged segments. We were able to analyze only pupal and adult phenotypes with a relatively moderate *Tm-sd* RNAi effect, because

severely affected larvae showed ecdysial arrest similar to that reported in the ladybird beetle, the mechanism of which is unknown (18). We observed significant defects in wing and elytra formation in *Tm-vg* and *Tm-sd* RNAi phenotypes, and the hypomeron regions were almost completely absent (Fig. 3, E and F, and fig. S4). Because of the lack of the hypomeron, these pupae and adults had round-shaped pronota compared with *egfp* dsRNA-injected beetles (Fig. 3, B, C, E, and F, versus A and D; and fig. S4). All seven pairs of denticular outgrowths were absent from the pupal abdomen in *Tm-vg* and *Tm-sd* RNAi beetles (Fig. 3, B and C, and fig. S4). No significant effects were detected in the adult abdomen, as the outgrowth is specific to pupae. These findings demonstrate significant roles of *Tm-vg* and *Tm-sd* in wing, prothoracic hypomeron, and pupal abdominal denticular outgrowth formation, which suggest serial homology among these structures because of a shared central developmental mechanism.

Fig. 2. *Tm-vg* and *Tm-sd* mRNA expression in developing hypomeron, wing, and the denticular outgrowth. (A) Schematic illustration of T1, T2, and A2 transverse section morphologies. (B to J) Expression analysis of *Tm-vg*. (K to S) Expression analysis of *Tm-sd*. Arrowheads indicate developing hypomeron, wing, and the denticular outgrowth in T1, T2, and A2, respectively. (E to G) and (N to P) show magnified image of boxed regions in (B to D) and (K to M), respectively. Sense riboprobes were used as negative controls (H to J and Q to S). Dotted line indicates developing primordia inside external cuticle.

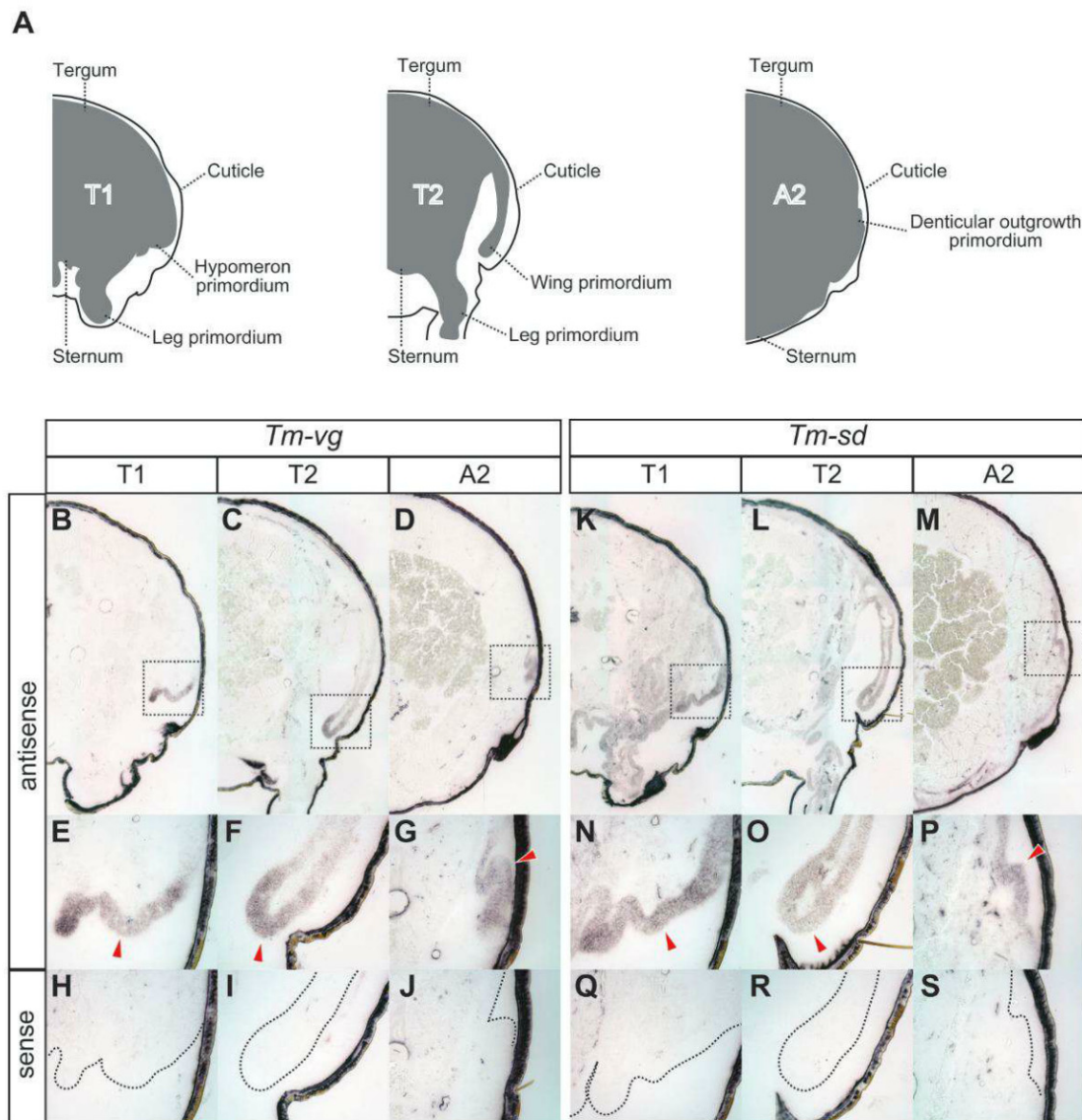
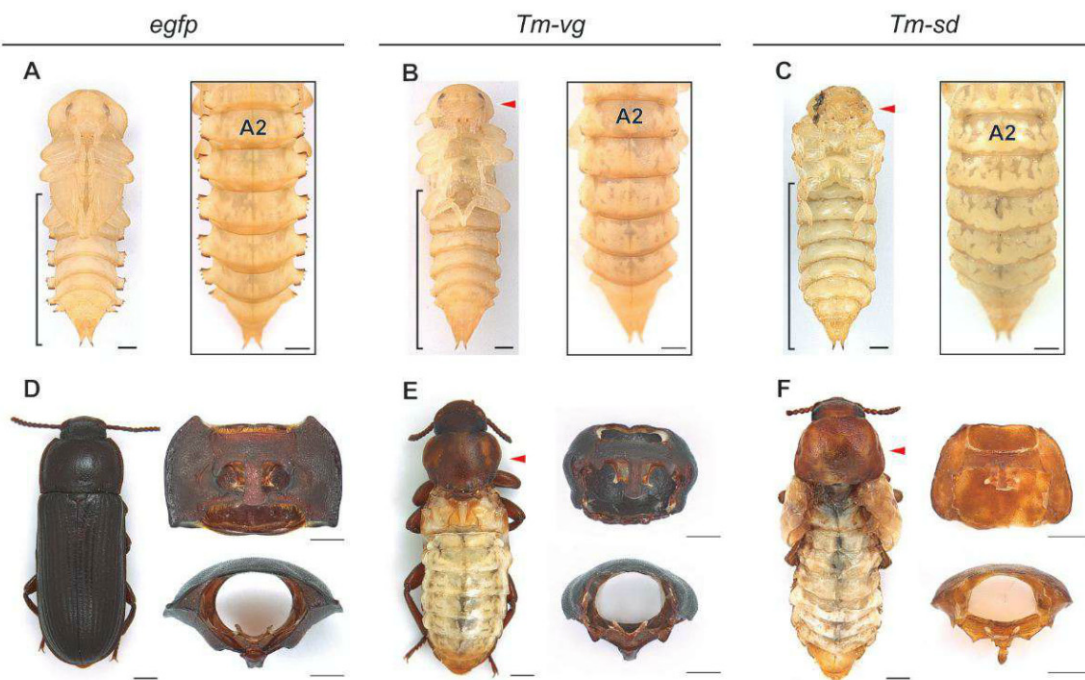


Fig. 3. *Tm-vg* and *Tm-sd* function in nonwing-tissue formation. (A to C) Phenotypes of *egfp* (A), *Tm-vg* (B) and *Tm-sd* (C) dsRNA-injected pupae shown in ventral view. *egfp* dsRNA was injected as a negative control. The denticular outgrowths were absent in RNAi beetles (bracket). (Right sides) A dorsal view of the pupal abdomen. (D to F) Adult phenotypes of *egfp* (D), *Tm-vg* (E) and *Tm-sd* (F) dsRNA-injected beetles shown in dorsal view. Arrowhead shows round-shaped prothorax. Dissected prothorax is shown in ventral (right sides, top) and caudal (right sides, bottom) views. Scale bar, 1 mm.



Our RNAi analysis provides the functional evidence for a central role of *vg* in the dorso-lateral extension of the body wall, which had been suggested by gene-expression analysis of the apterygote jumping bristletail (*Pedetontus unimaculatus*) (19). It raises the idea that WSHs might exist as segmental lateral outgrowths regulated by *Vg* and *Sd* in ancestral insects. We can find such lateral outgrowths in some fossil records (20, 21). Note that *snail*, a wing primordium marker, is detected in patches at the lateral position of winged (T2 and T3) and nonwinged (T1 and abdomen) segments in the embryo of *T. castaneum* (22). This *snail* expression also suggests the existence of primordial cells for WSHs, which have a shared developmental origin. Taken together, founder cells of WSHs might be first established in the embryonic stage and differentiate into diverse forms by *Hox*-modification of the *Vg* and *Sd* gene regulatory network in a segment-specific manner. Indeed, *Tm-Scr* and *Tm-Ubx* mRNAs were expressed during development of the hypomeron and the denticular outgrowth, respectively, in the middle of the prepupal stage (fig. S5), which suggested that *Hox* genes are conferring unique identities to these body “appendages.” Viewed in an evolutionary context, the independent evolution of *Hox* regulation targeting lateral outgrowths in each segment may have led to the morpholog-

ical diversification in a manner similar to the process underlying the diversification of ventral appendages (fig. S6).

WSHs with highly diverged forms might also exist in the nonwinged segments of other insect species. Indeed, it has been shown that treehopper’s prothoracic helmet could be a re-appearance of a form of dorsal appendage, although this idea is still controversial (23–25). In cockroaches, homology of the prothoracic paranotal region to the wing has been suggested (26–28).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1234219/DC1
Materials and Methods
Figs. S1 to S7
Table S1
Reference (29)

18 December 2012; accepted 28 February 2013
Published online 14 March 2013;
10.1126/science.1234219

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Science in Wales

The southwestern corner of the United Kingdom, with its population of just 3 million people, is making a bold bid for scientific greatness. In 2012, the government of Wales announced an agenda for scientific growth. The central program is built around recruiting research stars: high profile scientists who will move their laboratories to Wales and become part of national research networks. The program is funded with £50 million—nearly \$80 million. The Welsh government also wants to support science education, collaboration, and technology transfer, and quite simply, to be better at promoting Welsh science. Researchers are spreading the word about Wales as it launches an ambitious scientific plan. **By Chris Tachibana**

Cardiff Castle

Wales, known as a land of poets, artists, bards (and rugby), wants to be just as famous for its science. Benchmarking against the other parts of the United Kingdom—England, Northern Ireland, and Scotland—showed that although Wales has five percent of the U.K. population, it receives only about three percent of U.K. Research Council funding. Wales excels at attracting support for the arts, but could do better in science. This analysis prompted the government, in spring 2012, to release Science for Wales, a 60-page document with a five-year vision for Welsh science. The plan could support the Welsh economy by creating research jobs and businesses. A major goal is increasing the share of U.K. research funds to be proportional to the population by 2017.

Of course, research funding is not doled out based on population numbers, and must be earned. But Wales feels it deserves a bigger piece of the pie. “Scientific productivity is a point of pride for U.K. ministers, who say we produce more with less, and Welsh research has quite a few good examples of that,” says **Peter Halligan**, head of Strategic Futures at Higher Education Wales. “In several areas Wales does very well. In psychiatry and psychology we’re in the top 10 for citations per paper, but many people aren’t aware of that. Wales has made significant progress over the past decade and currently exceeds the world and European average for research impact despite producing proportionally fewer overall publications.” Computer science, social

science, engineering, and neuroscience are other areas where Wales is punching above its weight, says Halligan. And with the Science for Wales agenda, the Welsh government is trying to do more to develop that potential.

Driving the new scientific plan is **John Harries**, who became the first Welsh chief scientific adviser in 2010. “We’ve got some real pinnacles of excellence in Wales,” said Harries in a 2012 BBC interview, “but we need to augment the quality of our work.” Science for Wales, he said, “can up our game and bring more research money to Wales.”

The Centerpiece Of The Agenda

At the center of the Science for Wales agenda is *Sêr Cymru* or Stars Wales, funded by £50 million over five years from the government, plus university matching funds. *Sêr Cymru* aims to boost Welsh science by bringing a few internationally recognized researchers—the stars—to Welsh universities. The program is recruiting research chairs in each of three Grand Challenge areas: life sciences and health; low carbon, energy and environment; and advanced engineering and materials.

National Research Networks will be developed around each Grand Challenge area. Network directors **continued**

“Scientific productivity is a point of pride for U.K. ministers, who say we produce more with less, and Welsh research has quite a few good examples of that.”

—Peter Halligan

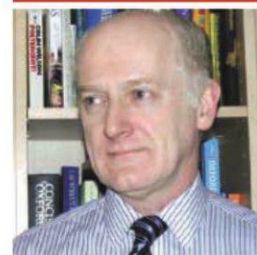
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Peter Halligan





“We [The Higher Education Funding Council for Wales] fund all areas for teachers with additional funding for science because of expenses such as labs.”

—David Blaney

but the program faces an issue familiar to any university recruiter: established scientists are reluctant to relocate. Mid- to late-career professors often have families and strong connections to their institutes and their communities. In addition, finding high-profile candidates in some fields, like engineering or low-carbon research, can be difficult because the most well-known science awards rarely go to researchers in those areas.

“Sêr Cymru is a fantastic idea,” says **Davey Jones**, who chairs Soil and Environmental Science at Bangor University, “but it’s been difficult to find people. The energy and environment fields don’t have a lot of Nobel Prize winners like they do in life sciences and health.” Setting up the National Research Networks has also been challenging because they must balance inclusiveness with becoming too diffuse, distributing resources equitably with boosting areas of strengths, and casting a wide net for contributors while connecting the most relevant collaborators. Says Jones, “We need to concentrate our efforts on fields where we have expertise. If we focus on the things we’re already good at, we can make them better.” An example, he says, is the Welsh government’s recent investment in regional agro-environmental activities. These efforts have increased interaction between universities, policymakers, and regulators around sustainable management of agricultural ecosystems, says Jones. “The science we do in this area makes a difference. It’s directly made things better for farmers.”

As the new science agenda gets under way, educators want to make sure that the emphasis on three areas of science and the focus on senior researchers does not neglect students and junior faculty. Sêr Cymru fits well with the country’s current research and education strategy,

will identify, encourage, and direct collaborative grants and projects in the priority research areas. First Minister of Wales **Carwyn Jones** says, “Here in Wales we certainly have a lot to be proud of, but we now need to build further on our science base to develop a dynamic and strong research community based on international excellence. That is what our Sêr Cymru program is about—attracting top scientific researchers and their teams to Wales.”

Installing preeminent researchers at Welsh universities could boost the Welsh science agenda, stimulating funding, publications, and further recruitment across the country. It’s a bold approach in times of general budget austerity,

says **David Blaney**, chief executive of the Higher Education Funding Council for Wales (HEFCW), the government arm that funds Wales’ universities and colleges. In fact, he says, by increasing agency efficiency, HEFCW was able to contribute £15 million to Sêr Cymru, “over and above” other research funding. Blaney says that although changes in government support for undergraduates recently reduced HEFCW funds, contributions to general research should remain steady at £71 million annually. In addition, he says, “We fund all areas for teachers—with additional funding for science because of expenses such as labs.” The government is working to promote science in the school curriculum, for example establishing a National Science Academy in 2010 to coordinate activities that encourage science education.

Other New Initiatives

Early- to mid-career researchers in Wales can find new opportunities for professional development and collaboration through Welsh Crucible. Participants in this program must show achievement in their field and commitment to a research career in Wales. The program is designed to support networking and collaboration, especially between scientists at different universities and in different fields. **Lijie Li** participated in the first round of Welsh Crucible in 2011. Li is a senior lecturer in the college of engineering at Swansea University who works on nanosensors and actuators that detect and respond to the environment. He says the program introduced him to researchers from other universities in Wales that he might not otherwise have met. The events, says Li, “allowed us to come together for opportunities to share ideas and discuss our research.”

Through Welsh Crucible, Li and his colleagues at Swansea University received seed funding for two collaborative projects with researchers at Cardiff University. The studies are small, says Li, but designed to gather preliminary data that could lead to additional funding. One project is focused on investigating ways to transfer mechanical movement into electrical energy, which could lead to environmentally friendly energy sources, for example for wearable devices. Li says Welsh Crucible has accomplished its goal of bringing Welsh researchers from multiple disciplines together, and his new collaborations are evidence. “I hope it will eventually expand to the rest of the U.K. and perhaps internationally,” he says.

Li’s office and laboratory are less than five miles from another major Welsh science project, the £400 million Swansea University Science and Innovation Campus. Leading the project is Pro Vice Chancellor **Iwan Davies**, who says the campus “represents a major scientific facility for Wales.” In addition to providing much-needed space and infrastructure at Swansea University, especially for engineering, Davies hopes the new campus “will attract the very best scholars to the university.”

The Welsh and U.K. governments and the Wales European Funding Office made substantial contributions to the first phase of the Science and Innovation Campus, projected to open in 2015. The oil company BP donated nearly 70 acres of land. The campus will incorporate a science park that will include the College of Engineering, **continued**

Higher Education Wales - is the representative body for Wales higher education institutions promoting university interests in Wales, UK and internationally.



Research standing - Welsh universities are part of a world-leading UK science base, second only to the US in its share of global citations. Over the past decade the number of citations per research paper from Welsh universities grew at a rate that overtook the UK average in the period 2006-2010.

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HEFCW is the largest single funder of research in Wales. It is also working with the Welsh Government to implement the Sêr Cymru programme which will provide additional investment for research in Wales.

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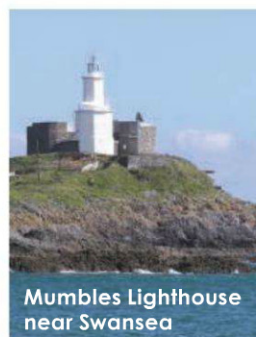
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Science for Wales
wales.gov.uk/docs/det/publications/120306scienceen.pdf

Sêr Cymru Program
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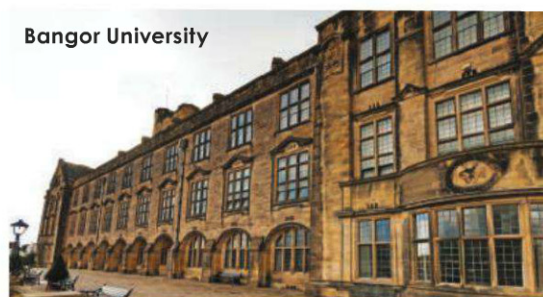
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Lijie Li



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the School of Business and Economics, and space for industry. For example, Swansea Materials and Research Testing (SMaRT), a subsidiary of the university, will be located at the new campus. SMaRT performs tests on materials for clients such as Rolls Royce, Dyson, and Airbus.

“Wales is naturally attractive to many industries. It has a history of high-quality engineering using traditional raw materials—steel and coal—and is now working in more advanced materials like high-performance composites,” says **Andrew R. Barron**. Barron has an endowed chair in chemistry at Rice University, an honorary chair in engineering at Swansea University, and is known for nonacademic research on vintage sports cars. His academic work covers the trio of focus areas in Science for Wales: materials engineering, life sciences, and environmental research. Barron interacted with Welsh industries and academic institutions as the first Prince of Wales Visiting Innovator in 2009. “At the time,” says Barron, “they were just getting relationships between companies and universities going. That has grown a lot in the last few years.” Sêr Cymru could drive job creation and better science education in Wales, predicts Barron, especially if there is growth in research and development as well as manufacturing.

The life sciences have high potential for expansion in Wales, especially since researchers have access to the data and tissue banks of the U.K. National Health Service. Barron, a featured speaker at the 2013 BioScience Wales conference, says that he used the National Health Service resources as a selling point when connecting a U.S. company that is developing a pancreatic cancer test with Welsh collaborators. Another international, life sciences facility in Wales is the Medical Research Council (MRC) Centre for Neuropsychiatric Genetics and Genomics. Housed at Cardiff University, the center capitalizes on Cardiff’s exceptional researchers and resources in neuroscience and

mental health. Recently, MRC scientists have made notable advances in areas such as Alzheimer’s disease, Huntington’s disease, schizophrenia, bipolar disorder, depression, and ADHD. An additional push for the life sciences industry comes from the Welsh government, which announced the Life Sciences Wales Investment Fund in May 2012. The government has pledged up to £100 million for the fund to stimulate life sciences research and development.

Opto-electronics (or photonics) is a £1 billion-per-year industry for Wales and an example of how a scientific strength can have a local and national economic impact, says **Paul Rees**, professor of optics: technology and metrology, Glyndŵr University. North Wales has the largest cluster of opto-electronics companies in the United Kingdom, says Rees, and the industry, community, and universities have “a symbiotic relationship.” University-industry connections and the relevant skills of local residents have made the region attractive to companies and strengthened the opportunities for opto-electronics research. The Welsh Opto-Electronics Forum, an industry-led interest group that originated in North Wales, is working with both industry and universities to respond to national initiatives such as Sêr Cymru. Also in the area is OpTIC Glyndŵr, part of Glyndŵr University, which includes incubator space for high-technology companies.” OpTIC Glyndŵr is a facilitator of exchange of expertise and academic-industrial partnerships, a door between academia and industry,” says Rees.

Combining Strengths

Conversations about how a small place like Wales, with three million people, can have an international scientific impact, often include the terms “collaborations,” “mergers,” and “critical mass.” The showcase example is frequently the Institute of Biological, **continued**

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Transforming our understanding of disease, helping save lives

Our Vision and Mission

Cellular research has an extraordinary potential to transform our understanding of disease and to save lives. Our vision is to help scientists worldwide make the advances that will bring this potential to life. We offer tools and solutions that help researchers better understand complex cellular problems. Our mission is to develop innovative cell technologies that fully embrace our heritage in biology, healthcare and engineering.

Making cell therapy a reality for patients

Cell therapy has the potential to help millions of patients suffering from life-threatening and life-limiting diseases like cancer and heart disease. GE is investing its broad expertise in biology, healthcare and engineering to address some of the biggest challenges in cell therapy.

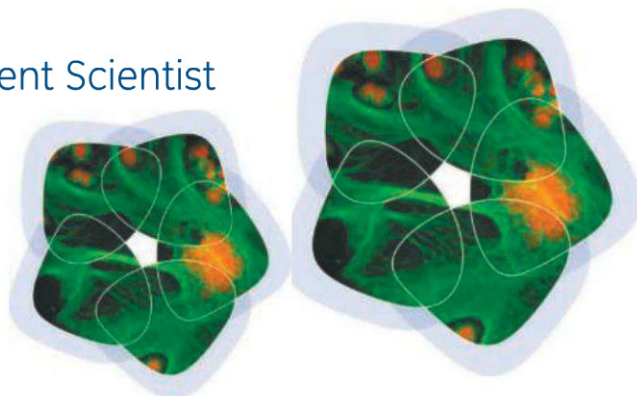
Biography of Key Staff : Stephen Minger

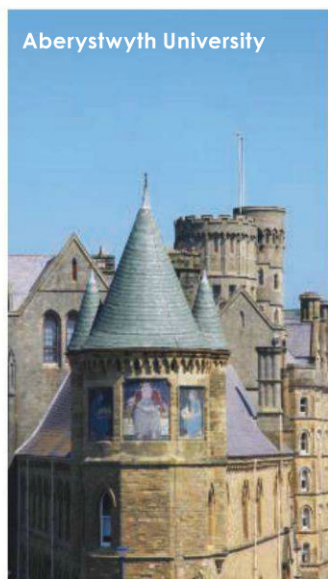
Dr. Stephen Minger is Chief Scientific Officer for the Research and Applied Markets business at GE Healthcare Life Sciences. Known globally as a leading stem cell scientist and as a high-profile advocate for the advancement of stem cell science, Dr. Minger is responsible for directing GE Healthcare Life Sciences' cell-based technologies for use in drug discovery and pharmaceutical research and enabling technologies for the rapidly emerging field of cell therapy.

We are recruiting and have the following open vacancies within GE Healthcare, Cardiff.

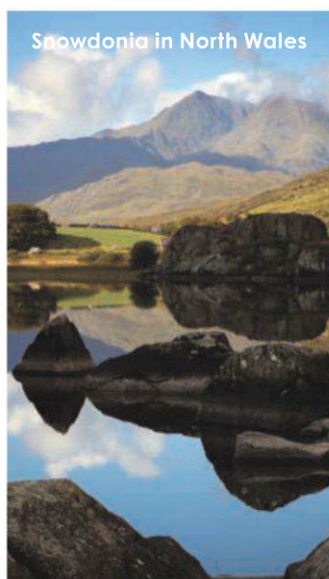
- **1726836** Senior Development Scientist/Immunologist
- **1706928** R&D Team Leader
- **1703822** Development/Senior Development Scientist

To apply please visit www.gecareers.com





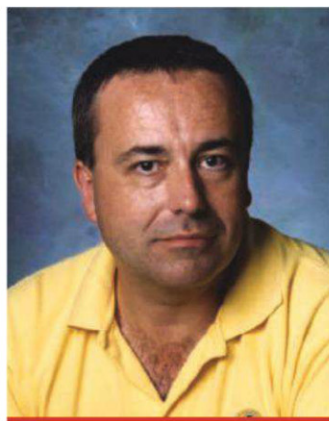
Aberystwyth University



Snowdonia in North Wales

“Wales is naturally attractive to many industries. It has a history of high-quality engineering using traditional raw materials—steel and coal—and is now working in more advanced materials like high-performance composites.”

—Andrew R. Barron



Environmental and Rural Sciences (IBERS). With 350 staff, 150 scientists, and 1,200 undergraduates, IBERS is a merger of the Institutes of Rural Sciences and Biological Sciences at Aberystwyth University and the Institute for Grassland and Environmental Research from the U.K.’s Biotechnology and Biological Science Research Council, with a formal collaboration with the Bangor University College of Natural Sciences. Aberystwyth University brings strengths in plant sciences and Bangor University has expertise in soil science, agroforestry, and social sciences, says IBERS Director **Wayne Powell**.

“By bringing together institutes and departments that were working in isolation before, we’ve created a center that attracts people who wouldn’t normally think of coming to Wales,” says Powell. Joint faculty appointments and collaborative grant applications have helped make the geographically dispersed groups into a single institution, he says. With a broad mission covering animal sciences to biodiversity, IBERS can influence the areas of climate change, sustainable and renewable

energy, food security, and others, says Powell. For example, IBERS is breeding grasses with high sugar content, suitable for grassland ecosystems in the United Kingdom and the United States. The economic and environmental benefits of using these grasses include efficient weight gain when ruminants are fed the high carbohydrate diet, with “fewer pollutants coming out their back end.” Waste from the grasses can be used for bioethanol production, sparing food sources such as maize. Powell says the high-tech grasses lead directly to products while supporting rural communities.

Expertise in energy and engineering is the foundation of the Low Carbon Research Institute (LCRI). This collaborative enterprise has representatives from six Welsh institutes and universities but is led and managed by Cardiff University, based on its strengths in energy research. **Alan Guwy**, head of the Sustainable Energy Research Centre, University of Glamorgan, leads research in hydrogen energy systems for the LCRI. This area of research covers hydrogen vehicles and fuel cells as well as biological production of hydrogen and use of its byproducts. “LCRI researchers have a history of working in the low carbon area. Now, in this consortium, we’re feeding off each other, developing new ideas, learning about funding opportunities, and meeting with funders and other academics around the world,” says Guwy. For instance, he explains, scientists at Glamorgan are working on biofermentation of hydrogen and methane from biowastes. Swansea University researchers contribute expertise in separation of wastes from valuable fermentation intermediates, and Bangor University collaborators focus on end products and testing.

The critical mass principle applies to Welsh universities, which have already gone through many mergers, name changes, and alliances, with more on the way. Researchers, administrators, and government officials alike agree that mergers are a good idea. The challenge is finding ways to combine forces in a way that strengthens the research and educational system, yet retains previous investments in infrastructure and preserves unique institutional cultures. Future mergers could result in the 11 Welsh universities coalescing into about eight. The universities understand the need to collaborate and coordinate. In 2009, Aberystwyth, Bangor, Cardiff, Swansea, and Glamorgan universities created the Saint David’s Day Group with a goal of reaching out to businesses, the government, and international contacts to help Wales out of the recession.

The central element of the Science for Wales agenda is under way, as universities search for Sêr Cymru research chairs and National Research Network directors. In the meantime, scientists in Wales are asked to consider hosting international conferences, serving on U.K. councils, and publicizing their research successes. It’s all part of getting the word out about science in Wales. At Aberystwyth University, Powell says, “It’s a good time to be considering Wales as a destination. We have a real opportunity now to combine scientific excellence and impact, all in a great environment to live.”

Chris Tachibana is a science writer based in Seattle, USA, and Copenhagen, Denmark.

DOI: 10.1126/science.opms.r1300133

Heal the sick, advance the science, share the knowledge.

Mayo Clinic in Rochester, MN is seeking a Cancer Immunology and Immunotherapy Researcher who will be expected to maintain a nationally/internationally recognized, extramurally funded program of research within the Department of Immunology, a basic science department within the College of Medicine, and be an active participant in the Mayo Clinic Cancer Center.

The Mayo Clinic is a collaborative environment, bringing researchers and clinicians together, to bring cutting-edge research from the bench to the bedside. Resources available at the Mayo Clinic include NIH-funded CTSA and T32 grants, NCI-funded Comprehensive Cancer Center and several active, funded SPORes in Ovarian Cancer, Breast Cancer, Pancreatic Cancer, Brain Cancer and Lymphoma.

A successful candidate will include doctoral degree in biomedical sciences (MD and/or PhD), national recognition and experience in the field and a strong track record of publication. Preference will be given to applicants with established research programs in any aspect of cancer immunology and immunotherapy, but individuals with active programs involving mobilizing the immune response in cancer are particularly encouraged to apply.

Mayo Clinic is an excellent choice for the candidate who is seeking a career in a world-class academic medical center that is consistently recognized by *U.S. News and World Report* as one of America's "Best Hospitals." Mayo Clinic's multidisciplinary group practice focuses on providing high-quality, compassionate medical care with a primary value that "the needs of the patient come first." Mayo Clinic is a nonprofit organization with approximately 3,800 physicians and scientists across all locations working in a unique environment that brings together the best in patient care, ground breaking research and innovative medical and graduate education.

Mayo Clinic offers a highly competitive compensation package, which includes exceptional benefits, and has been recognized by *Fortune* magazine as one of the "100 Best Companies to Work For."

To apply and learn more, please visit: www.mayoclinic.org/scientist-jobs/ and reference job posting #22631BR. Applicants should include a CV and a statement of research interests. Specific questions related to the job posting should be directed to:

Virginia Shapiro, Ph.D.
Immunology
Mayo Clinic
Email: helgren.brent@mayo.edu

Mayo Clinic is an affirmative action and equal opportunity employer. Post-offer/pre-employment screening is required.

The University Duisburg-Essen invites applications for the following position in the **Faculty of Medicine (University Hospital)** and the **Center for Medical Biotechnology (ZMB)**. The position is available from 1st January 2014:

University professorship (salary grade W 3 BBesG) for "Medical Biology"

The ideal candidate has profound expertise in basic research on the molecular mechanisms of cell physiology and its regulation in the field of Medical Biology. Investigators with a research focus on molecular disease mechanisms, preferentially concerning cancer biology, infectious diseases or immunology, are particularly encouraged to apply. Strong applications focusing on other fields that complement and extend the existing strengths of the medical faculty (<http://www.uni-due.de/med/en/index.php>) and the ZMB (<http://www.uni-due.de/zmb/research/index.shtml>) are also welcome. Cooperation with the local research clusters as well as their further development is highly desired.

Candidates are expected to run an independent extramurally funded, competitive research program and to participate in teaching within the Biology and Medical Biology programs.

According to §36 of the NRW Act (Institutions of Higher Learning) the requirements for this position are a university degree in the fields of medicine/bio-medicine, doctorate and additional scientific achievements attained in the context of a junior professorship, post-doctoral thesis or related scientific work at a university, research institution, or in another relevant area.

The University of Duisburg-Essen aims to increase diversity among its members (s. <http://uni-due.de/diversity>) and strives to raise the ratio of female scientific personal and thus especially encourages women with relevant qualifications to apply. Women with matching qualifications would receive preferential consideration. Applications from severely disabled candidates according to § 2 Abs. 3 SGB IX are welcome.

Applications, including the usual supporting documents (curriculum vitae, list of scientific publications, present and future research plans, information on teaching experience to date, involvement in academic self-administration and information about third-party funding) and the application form (available on <http://www.uni-due.de/zmb/application-w3-medbio>), should be submitted preferably by e mail within a period of 6 weeks after publication of this advertisement to the

Dekan der Fakultät für Medizin der Universität Duisburg-Essen, Univ.-Prof. Dr. Jan Buer, Dekanat der Medizinischen Fakultät, Hufelandstraße 55, 45122 Essen, Germany, e-mail: medizin-dekanat@uk-essen.de

Further information can be obtained from the head of the ZMB, Univ.-Prof. Dr. Michael Ehrmann, Tel. +49-201 183 2949, e-mail: michael.ehrmann@uni-due.de



FACULTY POSITION IN COASTAL HUMAN-ENVIRONMENT SYSTEMS Stanford University

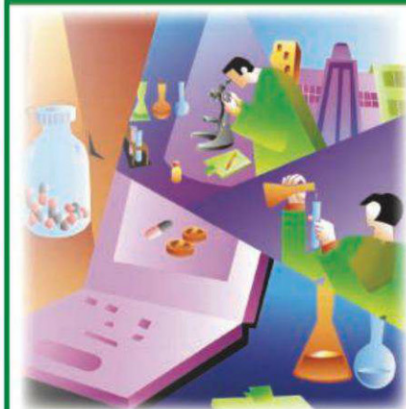
Stanford University seeks an innovative scholar to fill a tenure-track faculty position in the area of human-environment interactions in coastal zones. The successful candidate is expected to be an active participant in both the Department of Environmental Earth System Science (<https://pangea.stanford.edu/departments/eess/>) and the Center for Oceans Solutions (COS) in the Stanford Woods Institute for the Environment (<http://www.centerforoceansolutions.org/>). EESS is an interdisciplinary department with a research focus on current and future environmental problems. COS is a collaboration among Stanford University, the Monterey Bay Aquarium, and the Monterey Bay Aquarium Research Institute that works to solve the major problems facing the ocean and prepares leaders to take on those challenges.

The appointment will be joint between the Department of Environmental Earth System Science and the Stanford Woods Institute. The level of the appointment is open, with a preference for candidates at the junior rank. We seek a motivated, broad-thinking scholar whose research bridges the natural science and human dimensions of the coastal environment, with a focus on understanding how human and biophysical processes interact to affect the structure and function of marine and coastal systems. The successful candidate is expected to establish a vigorous research program that employs strong analytical methods. The successful candidate is also expected to teach classes and mentor graduate students in the Department of Environmental Earth System Science, to teach in the interdisciplinary environmental programs offered at Stanford (such as Earth Systems and the Emmett Interdisciplinary Program in Environment and Resources), and to be a leader in the broader oceans and marine communities at Stanford, including through active leadership of campus-wide interdisciplinary oceans initiatives. The position will involve research and teaching at Stanford's main campus, with close ties to Hopkins Marine Station and to COS partners and collaborators. Given the mission of COS, we are particularly interested in candidates with a desire to engage the public about scientific issues regarding coastal systems.

Applications containing a cover letter, curriculum vitae, and statements of research and teaching experience and interest should be included with your submission. Please apply at: <https://academicjobsonline.org/ajo/jobs/2644>. The search committee will request letters of recommendation for a subset of applicants following review of these materials. Review of applications will begin on June 1, 2013, and continue until the position is filled.

Stanford University is an Equal Opportunity Employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applications from women and minority groups, as well as others who would bring additional dimensions to the university's research, teaching and clinical missions.

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University of Connecticut
Health Center

Tenure-Track Immunology Faculty Position

The Department of Immunology at the University of Connecticut Health Center seeks an outstanding investigator for a tenure-track position at the Assistant/Associate/Full Professor level. Although all areas of immunology will be considered, we are particularly interested in individuals using molecular, cellular and translational approaches to study immune system function in vivo. Areas of priority include but are not limited to mucosal immunity including the microbiome, innate immunity, signal transduction and transcriptional control, and dendritic cell biology. The new hire will participate in a vibrant Ph.D. training program and have access to a growing translational research community. Salary and start-up funds are highly competitive and outstanding core facilities are available. Applicants must have a Ph.D. or M.D./Ph.D. and for senior appointments a history of sustained extramural funding and a high impact publication record. In addition to the beauty of the picturesque New England countryside, the Hartford area offers a vibrant arts and cultural scene and an exceptional outdoor sports environment.

Applicants should apply at <https://jobs.uchc.edu> search number 2012-067 and submit curriculum vitae, a two-page summary of research interests and the names of three references. Information may also be submitted to Dr. Leo Lefrançois, Ph.D., Chairman, Department of Immunology, UConn Health Center, Farmington, CT, 06030. Email: immunology@uchc.edu. For further information on UCHC immunology, please visit immune.uchc.edu.

UCHC is an Equal Opportunity Employer M/F/V/PwD



TEXAS TECH UNIVERSITY
HEALTH SCIENCES CENTER
at Amarillo

ASSISTANT/ASSOCIATE/FULL PROFESSOR POSITION IN PHARMACEUTICAL SCIENCES

The Department of Pharmaceutical Sciences at the Texas Tech University Health Sciences Center in Amarillo seeks applicants for a tenure-track faculty position. Applicants must have research experience in any aspect of pharmacology, medicinal chemistry, drug discovery and development, or drug delivery. The Department has 15 full-time faculty members with broad research interests in pharmaceutical sciences. The School of Pharmacy is located on the Amarillo regional campus, adjacent to the Schools of Medicine and Allied Health. Amarillo is a town of about 200,000 on the high plains of Texas with excellent opportunities for cultural activities, family life, and outdoor sports. For further information, visit our website: <http://www.ttuhsc.edu/sop/PharmSci/>.

Applicants must have earned a doctoral degree in pharmacology, medicinal chemistry, drug metabolism or related pharmaceutical sciences, with relevant postdoctoral experience. The candidates at the Assistant Professor level are expected to develop an extramurally funded research program, whereas candidates at the Associate or Full Professor level should have current peer-reviewed funding and a significant track record of publications. The successful candidate will teach in both professional pharmacy and graduate programs in the personal area of expertise, as well as mentor graduate (Ph.D. & M.S.) students and post-doctoral trainees. Competitive startup packages, incentives, and laboratory space are available. Please submit curriculum vitae, a summary of research and teaching interests, desired rank, and names and addresses of three references online at website: <http://jobs.texasstate.edu> (Job requisition #88407).

Screening and selection are set to begin **June 1, 2013**. For questions, contact the Search Committee Chair Dr. Ulrich Bickel (ulrich.bickel@ttuhsc.edu).

TTUHSC is an Equal Opportunity/Affirmative Action Institution. Minorities and women are encouraged to apply.

Imperial College London

Department of Life Sciences
Faculty of Natural Sciences

**Major Initiative in Cell Biology of
Model Organisms**

3 Lecturer/Senior Lecturer Appointments in Cell Biology of Model Organisms

Lecturer salary: £44,150 - £49,200 p.a.
Senior Lecturer minimum salary: £54,250 p.a.

We are seeking individuals who would welcome the opportunity to forge a career built around innovative approaches to research and excellence in teaching. We aim to make up to 3 academic appointments in Cell Biology in the Department of Life Sciences (<http://www3.imperial.ac.uk/lifesciences>) at the South Kensington Campus of Imperial College London. The Department offers extensive opportunities for collaboration within a world-class modern infrastructure. We are particularly interested in recruiting individuals who use model organisms to study fundamental or integrative cell biology, which may include developmental biology.

There is no restriction on the type of model organism (plant or animal) proposed for research programmes, which may have either pure or applied significance. You will have a strong publication record and demonstrable ability to direct a competitive independent research programme. You should have the drive and ambition to be among the best in your research field, and will also contribute to undergraduate and postgraduate teaching programmes. Whilst we anticipate that these appointments will be at Lecturer or Senior Lecturer level, there is potential for appointment at more senior levels for outstanding candidates.

For informal enquiries about the positions please contact Professors Murray Selkirk (m.selkirk@imperial.ac.uk), Nick Franks (n.franks@imperial.ac.uk) or Martin Buck (m.buck@imperial.ac.uk). For further information please see criteria detailed in the advertisement on our website.

Please apply online via our website <http://www3.imperial.ac.uk/employment> (please select "Job Search", enter the job title or vacancy reference number, including spaces, **NS 2013 071 DPN**, into "Keywords"). Please complete and upload an application form as directed.

Also please include your CV and a statement of research interests (up to two A4 pages). Should you have any queries about the application process please contact Sandrine Nurboja at s.nurboja@imperial.ac.uk.

Closing date: 31 May 2013.

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winner, a Stonewall Diversity Champion and a
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2014 Elite Nanyang Assistant Professorship

Nanyang Technological University (NTU), Singapore, invites outstanding young researchers and exceptional scholars to apply for appointment as a Nanyang Assistant Professor. Up to 10 appointments will be made.

Successful candidates will receive start-up research grant of up to SGD 1million (EUR 620K/ USD 800K/ JPY 79M) and an attractive remuneration package. They will hold tenure track appointments and play lead roles in NTU's multi-disciplinary, integrated research.

Candidates should be below 40 years of age, within 10 years of gaining their PhD or equivalent degree in their respective field, possess a few years of post-doctoral research experience, and ready to independently lead their own research groups.

For enquiries, email NanyangProfessorship@ntu.edu.sg

Applications now open for submission* at
www.ntu.edu.sg/nap till Sunday, 30 June 2013,
11:59pm (UTC / GMT +8:00)

**Kindly note that only online applications will be accepted.*

About Nanyang Technological University

A young, research-intensive university, Singapore's Nanyang Technological University (NTU) is one of the fastest-rising universities in the world. Helmed by Professor Bertil Andersson, winner of the Wilhelm Exner Medal, an honour bestowed on the world's best scientists, NTU is a melting pot of international award-winning scientists, young talents and eminent global partners. With its state-of-the-art facilities, NTU is building on its interdisciplinary strengths with cutting-edge research that improves lives and shapes the future. NTU now offers medicine at a new school jointly set up with Imperial College London.

www.ntu.edu.sg



TEXAS TECH UNIVERSITY
HEALTH SCIENCES CENTER
School of Medicine

**Professor and Chairperson
Department of Pharmacology and Neuroscience**

Texas Tech University Health Sciences Center School of Medicine invites applicants for the position of Chair of the Department of Pharmacology and Neuroscience. The new Chair will be expected to build new and cutting-edge research programs while maintaining the current research and teaching strengths of the department.

The Department's faculty research programs include neuroscience and substance abuse as well as cancer and cardiovascular pharmacology. The Department is also home to the South Plains Alcohol and Addiction Research Center (<http://www.ttuhsc.edu/centers/spaarc/>). There are currently 14 faculty in the department and 8 additional graduate faculty who provide graduate students additional research opportunities. Chair candidates must possess a Ph.D. and/or M.D. degree and be committed to directing, promoting, and developing the research and teaching missions of the department. Preferred candidates will have (a) a current senior-level academic rank (full professor or equivalent), (b) a strong extramurally-funded research program, and (c) excellent interpersonal skills and leadership qualities. TTUHSC has excellent core facilities for imaging, molecular biology and structural biology. Additional facilities are available in the Cancer Center and the Center for Membrane Protein Research in the TTUHSC School of Medicine and the Center for Biotechnology and Genomics in nearby Texas Tech University.

Lubbock is a city of over 200,000 on the South Plains of West Texas, with a family-friendly environment, good schools and a national ranking for a low cost of living and short average commute. The region has a diverse economy with strong contributions from agriculture, health care, and higher education. Lubbock is also home to Texas Tech University, which provides opportunities for collaboration as well as enjoyment of collegiate athletics and the performing and visual arts. Interested individuals must apply online at the following link: <https://jobs.texasstate.edu/postings/51691> (requisition number 88308). Women and underrepresented minorities are strongly encouraged to apply. CV and contact information for at least three references should be attached to the electronic application. Questions regarding the position can be directed to: **Luis Reuss, MD, Chair, Search Committee for Chair of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center, 3601 4th Street, Stop 6551, Lubbock, Texas 79430; E-Mail: Luis.Reuss@ttuhsc.edu**

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with #AAAS**



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**School of Biological Sciences
Nanyang Technological University, Singapore
Faculty positions in Immunology and Neurobiology**

The School of Biological Sciences (SBS) at Nanyang Technological University (NTU), Singapore, invites qualified academics with an international reputation in both education and research, to apply for two tenure-track positions as Professors in Immunology and Neurobiology.

The candidates are expected to undertake high quality internationally leading research in Immunology or Neurobiology in line with the research strategy of the School, obtain extramural research funding and supervise research staff and students. The candidates are also expected to contribute to, support and develop teaching and educational activity at undergraduate level, and supervise undergraduate students. The positions will be based full-time in Singapore. It will be an exciting opportunity for the candidates to lead in these two areas.

Salary will be competitive and will commensurate with qualifications and experience. The University offers a comprehensive fringe benefits package.

About The School

The School of Biological Sciences (SBS) was established in 2002 with the mission to make a strong contribution to Biological and Biomedical Sciences. Since then, many talented individuals from around the world and Singapore have joined SBS as Scientific Leaders, Researchers, PhD and Master students, working in the two Divisions of Structural Biology and Biochemistry as well as Molecular Genetics and Cell Biology. SBS research activities involve the areas of Proteomics, Genomics, Cell Biology, Molecular Biology, Infectious Disease, Immunology, Neurodegenerative Disease, Structural Biology and Biochemistry. It also collaborates with local and international research institutes, universities and hospitals, sharing the common goal to advance basic knowledge and translational applications in Biological and Biomedical Sciences. For further information about the School of Biological Sciences, please refer to the following website: <http://www.sbs.ntu.edu.sg/Pages/Home.aspx>.

To apply, please refer to the Guidelines for Submitting an Application for Faculty Appointment (<http://www.ntu.edu.sg/ohr/CareerOpportunities/SubmitanApplication/Pages/FacultyPositions.aspx>) and email your application (consisting of a cover letter, personal particulars form, curriculum vitae, concise statements of research interests and teaching plan, and the names and contact details of at least three referees) to: sbsrecruit-faculty@ntu.edu.sg, attention: **Professor Peter R Preiser (Acting Chair)**.

Enquiries about the positions can be addressed to the Acting Chair of School of Biological Sciences at: D-SBS@ntu.edu.sg.

Closing date for application is **31 May 2013** and shortlisted candidates are likely to be informed and interviewed in **Singapore from 19 to 21 June 2013**.

www.ntu.edu.sg

**IMB Investigatorship in Skin Biology
Institute of Medical Biology, A*STAR, Singapore**

The Institute of Medical Biology (IMB) is a government-funded research institute of Singapore's Agency for Science, Technology and Research (A*STAR). IMB's research covers Stem Cells, Genetic Diseases and Skin Biology. Our goal is to understand the determinants of human health and disease, develop novel therapeutic strategies, and move our research towards translated applications. See <http://www.imb.a-star.edu.sg>.

Skin Biology is a target growth area for A*STAR, and IMB leads a Skin Biology Cluster to promote alignment between its research and the interests of industry. IMB is launching a career development initiative for young researchers in skin biology/dermatology.

The **Investigator(s) in Skin Biology** will establish a research team on a research project aimed at understanding the biology and function of skin and its associated structures. Positions are open to clinicians and life scientists. The Investigator will be expected to collaborate with cross-disciplinary clinical and/or scientific partners. Funding is provided to cover the Investigator's salary, two junior staff positions, and laboratory running costs, initially for 3 years. The Investigator will also be expected to win external funding. Contracts are renewable for a 2nd term on evidence of excellent performance. Remuneration will be commensurate with qualifications and experience.

Submit applications to hr@imb.a-star.edu.sg, quoting "Investigatorship in Skin Biology" in the subject line. Applications must include a covering letter from the applicant, a full CV, a research proposal relevant to skin biology (max 1200 words), and the names and contact details of 3 academic referees. Closing date **15th May 2013**.

For further information about the Institute of Medical Biology, please see website at <http://www.imb.a-star.edu.sg>

Professor of Circuits and Systems Neuroinformatics

The Department of Information Technology and Electrical Engineering (www.ee.ethz.ch) at ETH Zurich and the Faculty of Science (www.mnf.uzh.ch) of the University of Zurich invite applications for the above-mentioned position in the Institute of Neuroinformatics (INI) to complement and extend its vigorous research activities.

The research of the candidate should be directed towards the theory and practice of computation in neural systems and behavior, with a strong interest in theory driven experimental neuroscience and information processing by neural circuits. The successful candidate will combine cutting-edge theories and experiments in neural information processing and computation to explore the causal links between neuronal circuits and behaviour in animals. An ability to develop requisite neurotechnologies will be an asset. The successful candidate will contribute to a highly collaborative multi-disciplinary environment. We encourage internationally recognized candidates with strong research records to apply. We seek to fill a full professorship position, but tenure track appointments will be considered as well. The new professor will be expected to teach undergraduate level courses (German or English) and graduate level courses (English).

The INI is a joint institute of the Faculty of Science of the University of Zurich and the Department of Information Technology and Electrical Engineering of ETH Zurich. The INI fosters research at the interface between neuroscience, computing and engineering through its research, teaching and graduate training, and specialist international workshop programs. Its members conduct a coordinated research program by means of multidisciplinary teams composed of about 70 biologists, physicists, psychologists, engineers and computer scientists. Through many levels of experiment and theory INI scientists explore how the circuits of the brain process information to generate intelligent behavior. They exploit new developments in silicon technology and computers as a means of developing models and hardware implementations of information processing and storage in the brain. In order to strengthen interdisciplinary research, the new professor should have a strong overlap with existing interests in the INI, and also forge links with other institutes of the University of Zurich and the ETH Zurich (for example: Biology, Brain Research, Pharmacology, Computer Science, Information Technology and Electrical Engineering, Mathematics, and Physics).

Applications should include a curriculum vitae, a list of publications and statements of future research and teaching activities. The letter of application should be addressed **to the President of ETH Zurich, Prof. Dr. Ralph Eichler. The closing date for applications is 15 June 2013.** ETH Zurich is an equal opportunity and affirmative action employer. In order to increase the number of women in leading academic positions, we specifically encourage women to apply. ETH Zurich is further responsive to the needs of dual career couples and qualifies as a family friendly employer. **Please apply online at www.facultyaffairs.ethz.ch.**

Imperial College London

Department of Life Sciences
Faculty of Natural Sciences

Lecturer/Senior Lecturer in Industrial Biotechnology

Lecturer salary: £44,150 - £49,200 p.a.
Senior Lecturer minimum salary: £54,250 p.a.

We are seeking an individual who would welcome the opportunity to forge a career built around innovative approaches to research and excellence in teaching. We aim to appoint a Lecturer or Senior Lecturer in Industrial Biotechnology in the Department of Life Sciences (<http://www3.imperial.ac.uk/lifesciences>) at the South Kensington Campus of Imperial College London. The Department offers extensive opportunities for collaboration within a world-class modern infrastructure.

The successful candidate could be working on any aspect of Industrial biotechnology such as Bioenergy, Metabolic engineering, or Synthetic biology. You will have a strong publication record and demonstrable ability to direct a competitive independent research programme. You should have the drive and ambition to be among the best in your research field, and will also contribute to undergraduate and postgraduate teaching programmes.

Informal enquiries about the position can be made by contacting Professor Martin Buck (m.buck@imperial.ac.uk). For further information please see criteria detailed in the advertisement on our website.

Please apply online via our website <http://www3.imperial.ac.uk/employment> (please select "Job Search", enter the job title or vacancy reference number, including spaces, **NS 2013 070 DPN**, into "Keywords"). Please complete and upload an application form as directed.

Also please include your CV and a statement of research interests (up to two A4 pages). Should you have any queries about the application process please contact Sandrine Nurboja at s.nurboja@imperial.ac.uk.

Closing date: 31 May 2013.



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UC DAVIS

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Applications: Application materials must be submitted via the website <https://recruit.ucdavis.edu/apply/JPF00055>. Additional inquiries should be directed to **Professor Russ Hovey, Chair of the Recruitment Advisory Committee**, 2145 Meyer Hall, Department of Animal Science, One Shields Avenue, University of California, Davis, CA 95616, (530) 752-1282; rchovey@ucdavis.edu. The position will remain open until filled. To ensure consideration, applications should be received by **June 1, 2013**.

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Smithsonian
National Museum of Natural History

RESEARCH ZOOLOGIST
Department of Vertebrate Zoology
National Museum of Natural History
Smithsonian Institution

The Smithsonian's National Museum of Natural History seeks a zoologist to conduct an integrative, specimen- or collection-based research program in vertebrate evolution and biodiversity (herpetology, ichthyology, mammalogy, and/or ornithology). The successful candidate is expected to develop an internationally recognized research program that makes important contributions to understanding vertebrate evolution and biodiversity through integrative research involving phylogenetics, anatomy, development, genomics, biogeography, conservation, informatics, or related fields. Frequent publication of highly regarded papers in competitive, peer-reviewed journals, curation of collections in specialty area, service to the scientific community in leadership capacities, acquisition of external funding, engagement in outreach activities, and mentorship of students are expected.

Full-time four-year term appointment with full Government benefits to be filled at the GS-12 level; U.S. citizenship required. The museum's authorized salary range for this position at this time is \$74,872 - \$79,864 per annum. College transcripts and proof of U.S. accreditation for foreign study must be submitted online by the closing date of announcement or your application will be disqualified. For complete requirements and application procedures, go to websites: <http://www.sih.si.edu> or <http://www.usajobs.gov> and refer to **Announcement 13A-JW-298398-DEU-NMNH**. The announcement opens April 22, 2013. Applications and all supporting documentation must be received on-line by June 3, 2013 and must reference the announcement number. *All applicants will be notified by e-mail when their application is received. The Smithsonian Institution is an Equal Opportunity Employer.*

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